PATENTS
Attorney Docket Number MPI1994-008CP2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#21

In re:	U.S. Patent No. 5,780,454
For:	**Boronic Ester and Acid Compounds**
Inventors:	Julian Adams, Yu-Ting Ma, Ross Stein, Matthew Baevsky, Louis Grenier, and Louis Plamondon
Assignee:	Millennium Pharmaceuticals, Inc.
Issued:	July 14, 1998

BY HAND DELIVERY

Mail Stop **Patent Ext.**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR EXTENSION OF PATENT TERM PURSUANT TO 35 U.S.C. § 156 AND 37 C.F.R. § 1.740

Pursuant to 35 U.S.C. § 156 and 37 C.F.R. § 1.740, Millennium Pharmaceuticals, Inc. ("Applicant") hereby applies for an extension of U.S. Patent No. 5,780,454 (the "'454 Patent"). Applicant is the assignee of the '454 patent. Copies of the assignment documents establishing ownership in Applicant are attached hereto as Exhibit A. A Power of Attorney granting authority to the undersigned registered practitioner to act on behalf of Applicant is attached hereto as Exhibit B.

Applicant provides the following information in fulfillment of the requirements of 37 C.F.R. § 1.740(a):

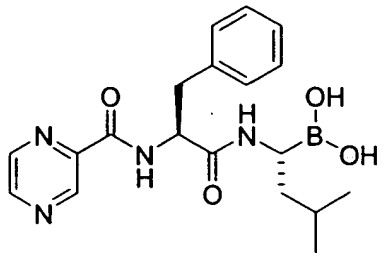
(1) **A complete identification of the approved product as by appropriate chemical and generic name, physical structure or characteristics;**

VELCADE™ (bortezomib) for Injection is an antineoplastic agent available for intravenous injection (IV) use only. Each single dose vial contains 3.5 mg of bortezomib as a sterile lyophilized powder. Inactive ingredient: 35 mg mannitol, USP.

Bortezomib is a modified dipeptidyl boronic acid. The product is provided as a mannitol boronic ester, which, in reconstituted form, consists of the mannitol ester in equilibrium with its hydrolysis product, the monomeric boronic acid. The drug substance exists in its cyclic anhydride form as a trimeric boroxine.

The chemical name for bortezomib, the monomeric boronic acid is [(1R)-3-methyl-1-[[(2S)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl)amino]propyl]amino]-butyl]boronic acid.

Bortezomib has the following chemical structure:



The molecular weight is 384.24. The molecular formula is $C_{19}H_{25}BN_4O_4$. The solubility of bortezomib, as the monomeric boronic acid, in water is 3.3-3.8 mg/mL in a pH range of 2-6.5.

(2) A complete identification of the Federal statute including the applicable provision of law under which the regulatory review occurred;

The regulatory review occurred under Section 505 of the Federal Food, Drug and Cosmetic Act ("FFDCA"), 21 U.S.C. § 301 et. seq.

(3) An identification of the date on which the product received permission for commercial marketing or use under the provision of law under which the applicable regulatory review period occurred;

VELCADE™ (bortezomib) for Injection was approved by FDA for commercial marketing on May 13, 2003.

(4) **In the case of a drug product, an identification of each active ingredient in the product and as to each active ingredient, a statement that it has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act, or a statement of when the active ingredient was approved for commercial marketing or use (either alone or in combination with other active ingredients), the use for which it was approved, and the provision of law under which it was approved;**

The sole active ingredient of the approved new drug (which is a human drug) is bortezomib, as described in response to (1) above. This active ingredient has not previously been approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act.

(5) **A statement that the application is being submitted within the sixty day period permitted for submission pursuant to § 1.720(f) and an identification of the date of the last day on which the application could be submitted;**

This application is being submitted within the sixty day period permitted for submission pursuant to 37 C.F.R. § 1.720(f). July 11, 2003 is the last day on which the application could be submitted.

(6) **A complete identification of the patent for which an extension is being sought by the name of the inventor, the patent number, the date of issue, and the date of expiration;**

A complete identification of the patent for which an extension is being sought is presented as follows:

Names of the Inventors:	Julian Adams; Yu-Ting Ma; Ross Stein; Matthew Baevsky; Louis Grenier; and Louis Plamondon
Patent Number:	5,780,454
Issue Date:	July 14, 1998
Date of Original Expiration:	October 28, 2014

(7) A copy of the patent for which an extension is being sought, including the entire specification (including claims) and drawings;

A copy of the '454 Patent is attached hereto as Exhibit C.

(8) A copy of any disclaimer, certificate of correction, receipt of maintenance fee payment, or reexamination certificate issued in the patent;

A copy of the PTO Maintenance Fee Statement is attached as Exhibit D.

No Statutory Disclaimer, Certificate of Correction, or Reexamination Certificate has ever issued in the '454 Patent.

(9) A statement that the patent claims the approved product or a method of using or manufacturing the approved product, and a showing which lists each applicable patent claim and demonstrates the manner in which one such patent claim reads on:

(i) The approved product, if the listed claims include any claim to the approved product;

The '454 Patent claims the approved product. The '454 Patent includes 22 claims, of which claims 1-13 and 15-22 read on the approved product. A claim chart that lists each applicable claim of the '454 Patent and demonstrates the manner in which claim 1 reads on the approved product is attached as Exhibit E.

(10) A statement beginning on a new page of the relevant dates and information pursuant to 35 U.S.C. § 156(g) in order to enable the Secretary of Health and Human Services or the Secretary of Agriculture, as appropriate, to determine the applicable regulatory review period as follows:

(i) For a patent claiming a human drug, antibiotic, or human biological product:

(A) The effective date of the investigational new drug (IND) application and the IND number;

IND # 56,515 was submitted on July 24, 1998, and became effective on August 22, 1998.

(B) The date on which a new drug application (NDA) or a Product License Application (PLA) was initially submitted and the NDA or PLA number; and

NDA # 21-602 was submitted on January 21, 2003.

(C) The date on which the NDA was approved or the Product License issued;

NDA #21-602 was approved on May 13, 2003.

(11) A brief description beginning on a new page of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities;

The required description and identification of dates is provided in Exhibit F attached hereto.

(12) A statement beginning on a new page that in the opinion of the applicant the patent is eligible for the extension and a statement as to the length of the extension claimed, including how the length of extension was determined;

In the opinion of Applicant, the '454 Patent is eligible for extension of patent term pursuant to 35 U.S.C. § 156(a) for the following reasons:

(1) The term of the '454 Patent has not expired before submission of this application.

(2) The term of the '454 Patent has never been extended.

(3) This application for patent term extension is being submitted by Millennium Pharmaceuticals, Inc., the record owner of the '454 Patent.

(4) Bortezomib was subject to a regulatory review period before its commercial marketing or use, as evident from Paragraph 11 above.

(5) The permission for the commercial marketing or use of bortezomib after such regulatory review period is the first commercial marketing or use of the product under the FFDCA.

Applicant contends that the '454 Patent is eligible for an extension of 920 days to May 5, 2017. The length of the asserted extension was calculated as follows:

(a) The post-IND/pre-approval period from August 22, 1998 to January 21, 2003 comprises 1,614 days (including 1 extra day for the leap year in 2000). The full extent of this period was subsequent to the patent issue date and is supported by the diligence showing in response to (11) above.

(b) The review period from January 21, 2003 to May 13, 2003 comprises 113 days. Applicant's diligence during the review period also is evident from the response to (11) above.

(c) One half of the testing period is 807 days.

(d) The sum of the review period and one-half of the testing period is 920 days ("modified regulatory review period").

(e) The original expiration date of the '454 Patent is October 28, 2014.

(f) Addition of the modified regulatory review period of 920 days recited under (d) to the original expiration date would extend the expiration date to May 5, 2017.

(g) The extension period is subject to a five year limitation under 35 U.S.C. § 156(g)(6)(A); hence, the '454 Patent cannot be extended beyond October 28, 2019.

(h) Pursuant to 35 U.S.C. § 156(c)(3), the extended term of the patent cannot exceed 14 years from the date of product approval; hence, the '454 Patent cannot be extended beyond May 13, 2017.

(i) In light of the conclusions stated in (f), (g), and (h), the extended expiration date of the '454 Patent is believed to be May 5, 2017. Therefore, the asserted extension for the '454 Patent is 920 days, from October 28, 2014 through May 5, 2017.

(13) A statement that applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought;

Applicant acknowledges its duty to disclose to the Commissioner of Patents and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

(14) The prescribed fee for receiving and acting upon the application for extension (see § 1.20(j)); and

Authorization is hereby given to charge the required petition fee of $1,120.00 to Deposit Account No. 501668. If this fee is insufficient or if any other fees are due for filing and processing of this application, authorization is hereby given to charge such fees to Deposit Account No. 501668.

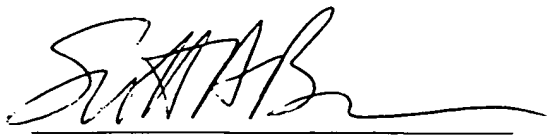
(15) The name, address, and telephone number of the person to whom inquiries and correspondence relating to the application for patent term extension are to be directed.

Inquiries and correspondence relating to this application for patent term extension are to be directed to:

Janice M. Klunder, Ph.D., Reg. No. 41,121
Intellectual Property Department
Millennium Pharmaceuticals, Inc.
75 Sidney Street
Cambridge, MA 02139
Phone: (617) 551-3699
Fax: (617) 374-0074
E-mail: jklunder@mpi.com

On the basis of the information provided herein, Applicant asserts that the '454 Patent is entitled to the requested 920 day extension of its term to May 5, 2017. Prompt action on this application is requested.

Respectfully submitted,



Scott A. Brown
Attorney for Applicant

Reg. No. 32,724
Intellectual Property Department
Millennium Pharmaceuticals, Inc.
75 Sidney Street
Cambridge, MA 02139
Phone: (617) 551-8662
Fax: (617) 374-0074
E-mail: scbrown@mpi.com

July _____, 2003

A

UNITED . .TES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

FEBRUARY 24, 1997

PTAS

STERNE, KESSLER, GOLDSTEIN & FOX
JOHN M. COVERT
1100 NEW YORK AVE., N.W.
SUITE 600
WASHINGTON, D.C. 20005-3934

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

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PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, NORTH TOWER BUILDING, SUITE 10C35, WASHINGTON, D.C. 20231.

RECORDATION DATE: 04/09/1996

REEL/FRAME: 7882/0067
NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
ADAMS, JULIAN — DOC DATE: 03/01/1996

ASSIGNOR:
MA, YU-TING — DOC DATE: 03/09/1996

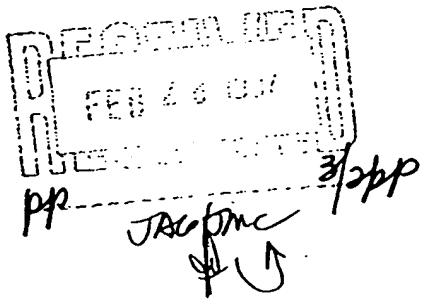
ASSIGNOR:
STEIN, ROSS — DOC DATE: 02/29/1996

ASSIGNOR:
BAEVSKY, MATTHEW — DOC DATE: 02/23/1996

ASSIGNOR:
GRENIER, LOUIS — DOC DATE: 02/29/1996

ASSIGNOR:
PLAMONDON, LOUIS — DOC DATE: 02/29/1996

ASSIGNEE:
PROSCRIPT, INC.
38 SIDNEY STREET
CAMBRIDGE, MASSACHUSETTS 02139



SERIAL NUMBER: 08549318
PATENT NUMBER:

FILING DATE: 10/27/1995
ISSUE DATE:

DIANE RUSSELE, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

04-15-1996
100173398

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APR 09 1996
RECEIPT ACCTNG. DIV.

581/40 4/9/96

U.S. Department of Commerce
Patent and Trademark Office

RECORDATION FORM COVER SHEET
PATENTS ONLY

To the Honorable Commissioner of Patents and Trademarks. Please record the attached original documents or copy thereof.

1. Name of conveying party(ies):

ADAMS, Julian
MA, Yu-Ting
STEIN, Ross
BAEVSKY, Matthew
GRENIER, Louis
PLAMONDON, Louis

Additional name(s) of conveying party(ies) attached? ☐ yes ☒ no

2. Name and address of receiving party(ies):

Name: ProScript, Inc.

Street Address: 38 Sidney Street

City: Cambridge State: Massachusetts Zip Code: 02139

Country: USA

Additional name(s) & address(es) attached? ☐ yes ☒ no

3. Nature of Conveyance:

☒ Assignment ☐ Merger
☐ Security Agreement ☐ Change of Name
☐ Other _____

Execution Date(s): March 1, 1996, February 29, 1996, February 29, 1996, February 29, 1996, March 9, 1996 and February 23, 1996

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is

A. Patent Application No(s)	B. Patent No(s)
08/549,318	

Additional numbers attached? ☐ yes ☒ no

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: **Sterne, Kessler, Goldstein & Fox P.L.L.C.**

Internal Address: c/o

Street Address: 1100 New York Ave., N.W.
Suite 600

City: Washington State: D.C. Zip Code: 20005-3934

6. Total number of applications and patents involved
One (1)

7. Total fee (37 C.F.R. § 3.41).....$ 40.00

☒ Enclosed

☐ Authorized to be charged to Deposit Account

8. Deposit Account Number: 19-0036

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9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

John M. Covert
John M. Covert
Registration No. 33,883

April 9, 1996
Date

040 JS 04/09/96 00549318

1 581 40.00 CK

Total number of pages including cover sheet, attachments and document Four (4)

OMB NO. 0651-0011 (exp.4/94)

Mail documents to be recorded with required cover sheet information to:
Commissioner of Patents and Trademarks, Box Assignments
Washington, D.C. 20231

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ASSIGNMENT

In consideration of the sum of One Dollar ($1.00) or equivalent and other good and valuable consideration paid to each of the undersigned: Julian ADAMS, Yu-Ting MA, Ross STEIN, Matthew BAEVSKY, Louis GRENIER and Louis PLAMONDON, the undersigned hereby sell(s) and assign(s) to ProScript, Inc. (the Assignee) their entire right, title and interest

check applicable box(es) ☒ for the United States of America (as defined in 35 U.S.C. § 100),
☒ and throughout the world,

in the invention(s) known as Boronic Ester and Acid Compounds, Synthesis and Uses for which application(s) for patent in the United States of America has (have) been executed by the undersigned on X 3/1/96 2/29/96 2/29/96; 2/29/96 (also known as United States Application No. 08/549,318, filed October 27, 1995), in any and all applications thereon, in any and all Letters Patent(s) therefor, and in any and all reissues, extensions, renewals, reexaminations of such applications or Letters Patent(s) and divisionals and continuing applications thereof to the full end of the term or terms for which such Letters Patent(s) issue, such entire right, title and interest to be held and enjoyed by the above-named Assignee to the same extent as they would have been held and enjoyed by the undersigned had this assignment and sale not been made.

The undersigned agree(s) to execute all papers necessary in connection with the application(s) and any continuing, divisional, reissue, reexamination or corresponding application(s) thereof and also to execute separate assignments in connection with such applications as the Assignee may deem necessary or expedient.

The undersigned agree(s) to execute all papers necessary in connection with any interference that may be declared concerning the application(s) or any continuing, divisional, reissue or reexamination application thereof and to cooperate with the Assignee in every way possible in obtaining evidence and going forward with such interference.

The undersigned hereby represents that undersigned has (have) full right to convey undersigned entire interest herein assigned, and that undersigned has (have) not executed, and will not execute, any agreement in conflict therewith.

The undersigned hereby grant(s) Robert Greene Sterne, Esquire, Registration No. 28,912, Edward J. Kessler, Esquire, Registration No. 25,688, Jorge A. Goldstein, Esquire, Registration No. 29,021, Samuel L. Fox, Esquire, Registration No. 30,353, David K.S. Cornwell, Esquire, Registration No. 31,944, Robert W. Esmond, Esquire, Registration No. 32,893, Tracy-Gene G. Durkin, Esquire, Registration No. 32,831, Michele A. Cimbala, Esquire, Registration No. 33,851, Michael B. Ray, Esquire, Registration No. 33,997 and Robert E. Sokohl, Registration No. 36,013 of STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C. 1100 New York Avenue, N.W., Suite 600, Washington, D.C. 20005-3934, power to insert in this assignment any further identification that may be necessary or desirable in order to comply with the rules of the United States Patent and Trademark Office for recordation of this document.

IN WITNESS WHEREOF, executed by the undersigned on the date(s) opposite their name(s).

Date: 3/1/96 Signature of Inventor: _____
Julian ADAMS

Date: _____ Signature of Inventor: _____
Yu-Ting MA

Date: 2/29/96 Signature of Inventor: _____
Ross STEIN

Date: _____ Signature of Inventor: _____
Matthew BAEVSKY

Date: 2/29/96 Signature of Inventor: _____
Louis GRENIER

Date: 2/29/96 Signature of Inventor: _____
Louis PLAMONDON

015.PTO
SKGF Rev. 2/96

ASSIGNMENT

In consideration of the sum of One Dollar ($1.00) or equivalent and other good and valuable consideration paid to each of the undersigned: Julian ADAMS, Yu-Ting MA, Ross STEIN, Matthew BAEVSKY, Louis GRENIER and Louis PLAMONDON, the undersigned hereby sell(s) and assign(s) to ProScript, Inc. (the Assignee) their entire right, title and interest

check applicable box(es) ☒ for the United States of America (as defined in 35 U.S.C. § 100),
☒ and throughout the world,

in the invention(s) known as Boronic Ester and Acid Compounds, Synthesis and Uses for which application(s) for patent in the United States of America has (have) been executed by the undersigned on X March 9, 1996 (also known as United States Application No. 08/549,318, filed October 27, 1995), in any and all applications thereon, in any and all Letters Patent(s) therefor, and in any and all reissues, extensions, renewals, reexaminations of such applications or Letters Patent(s) and divisionals and continuing applications thereof to the full end of the term or terms for which such Letters Patent(s) issue, such entire right, title and interest to be held and enjoyed by the above-named Assignee to the same extent as they would have been held and enjoyed by the undersigned had this assignment and sale not been made.

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IN WITNESS WHEREOF, executed by the undersigned on the date(s) opposite their name(s).

Date: _____ Signature of Inventor: _____
Julian ADAMS

Date: March 9, 1996 Signature of Inventor: _____
Yu-Ting MA

Date: _____ Signature of Inventor: _____
Ross STEIN

Date: _____ Signature of Inventor: _____
Matthew BAEVSKY

Date: _____ Signature of Inventor: _____
Louis GRENIER

Date: _____ Signature of Inventor: _____
Louis PLAMONDON

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check applicable box(es) ☒ for the United States of America (as defined in 35 U.S.C. § 100),
☒ and throughout the world,

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IN WITNESS WHEREOF, executed by the undersigned on the date(s) opposite their name(s).

Date: _____ Signature of Inventor: _____
Julian ADAMS

Date: _____ Signature of Inventor: _____
Yu-Ting MA

Date: _____ Signature of Inventor: _____
Ross STEIN

Date: 23 Feb 1996 Signature of Inventor: Matthew F. Baevsky 2/23/96
Matthew BAEVSKY

Date: _____ Signature of Inventor: _____
Louis GRENIER

Date: _____ Signature of Inventor: _____
Louis PLAMONDON

UNITED STATES PATENT AND TRADEMARK OFFICE

MARCH 08, 2002

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Chief Information Officer
Washington, DC 20231
www.uspto.gov

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JANICE M. KLUNDER, PH.D.
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BOSTON, MA 02109

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RECORDATION DATE: 01/10/2002

REEL/FRAME: 012454/0224
NUMBER OF PAGES: 5

BRIEF: MERGER (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
PROSCRIPT, INC., A DELAWARE CORPORATION

DOC DATE: 03/15/2000

ASSIGNEE:
LEUKOSITE, INC.
215 FIRST STREET
A DELAWARE CORPORATION
CAMBRIDGE, MASSACHUSETTS 02142

SERIAL NUMBER: 08549318
PATENT NUMBER: 5780454

FILING DATE: 10/27/1995
ISSUE DATE: 07/14/1998

MARY BENTON, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

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RE: 103576.135 US3
Action Date:
Action to be Taken:
Docketed by: AA 3/14/02

FORM PTO-1619A
Expires 06/30/99
OMB 0651-0027

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JAN 10 2002

01-16-2002

101951034

1.1002

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Conveying Party(ies)

- [ ] Mark if additional names of conveying parties attached

Name (line 1): ProScript, Inc.
Execution Date (Month Day Year): 03 15 00

Name (line 2): a Delaware Corporation

Second Party
Name (line 1):
Execution Date (Month Day Year):

Name (line 2):

Receiving Party

- [ ] Mark if additional names of receiving parties attached

Name (line 1): LeukoSite, Inc.

Name (line 2): a Delaware Corporation

Address (line 1): 215 First Street

Address (line 2):

Address (line 3): Cambridge (City) MA/USA (State/Country) 02142 (Zip Code)

- [ ] If document to be recorded is an assignment and the receiving party is not domiciled in the United States, an appointment of a domestic representative is attached. *(Designation must be a separate document from Assignment.)*

Domestic Representative Name and Address

Enter for the first Receiving Party only.

Name:

Address (line 1):

Address (line 2):

Address (line 3):

Address (line 4):

01/16/2002 AAHMED1 00000075 080219 5780454
01 FC:581 40.00 CH

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Expires 06/30/0[illegible]
OMB 0651-00[illegible]

U.S. Department of Commerce
Patent and Trademark Office
PATENT

Correspondent Name and Address

Area Code and Telephone Number: 617/526-6771

Name: Janice M. Klunder, Ph.D.

Address (line 1): Hale and Dorr LLP

Address (line 2): 60 State Street

Address (line 3):

Address (line 4): Boston, MA 02109

Pages

Enter the total number of pages of the attached conveyance document including any attachments. # 3

Application Number(s) or Patent Number(s)

☐ Mark if additional numbers attached

Enter either the Patent Application Number or the Patent Number (DO NOT ENTER BOTH numbers for the same property).

Patent Application Number(s)			Patent Number(s)		
			5780454		

If this document is being filed together with a new Patent Application, enter the date the patent application was signed by the first named executing inventor. Month Day Year

Patent Cooperation Treaty (PCT)

Enter PCT application number only if a U.S. Application Number has not been assigned.

PCT PCT PCT
PCT PCT PCT

Number of Properties

Enter the total number of properties involved. # 1

Fee Amount

Fee Amount for Properties Listed (37 CFR 3.41): $ 40.00

Method of Payment: Enclosed ☐ Deposit Account ☑

Deposit Account

(Enter for payment by deposit account or if additional fees can be charged to the account.)

Deposit Account Number: # 08-0219

Authorization to charge additional fees: Yes ☑ No ☐

Statement and Signature

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document. Charges to deposit account are authorized, as indicated herein.

Janice M. Klunder, Ph.D., Reg. No. 41,121	Janice M. Klunder	11/27/01
Name of Person Signing	Signature	Date

State of Delaware

Office of the Secretary of State

I, EDWARD J. FREEL, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF OWNERSHIP, WHICH MERGES:

"PROSCRIPT, INC.", A DELAWARE CORPORATION,

WITH AND INTO "LEUKOSITE, INC." UNDER THE NAME OF "LEUKOSITE, INC.", A CORPORATION ORGANIZED AND EXISTING UNDER THE LAWS OF THE STATE OF DELAWARE, AS RECEIVED AND FILED IN THIS OFFICE THE SIXTEENTH DAY OF MARCH, A.D. 2000, AT 12 O'CLOCK P.M.

Edward J. Freel, Secretary of State

2296418 8100M

001315321

AUTHENTICATION: 0512438

DATE: 06-21-00

CERTIFICATE OF OWNERSHIP AND MERGER

MERGING

ProScript, Inc.
(a Delaware corporation)

INTO

LeukoSite, Inc.
(a Delaware corporation)

LeukoSite, a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware (the "Corporation"), does hereby certify:

FIRST: That the Corporation was incorporated on the 1st day of May, 1992 pursuant to the General Corporation Law of the State of Delaware.

SECOND: That the Corporation owns all of the outstanding shares of each class of the stock of ProScript, Inc., a corporation incorporated on the 20th day of August, 1992 pursuant to the General Corporation Law of the State of Delaware.

THIRD: That the Board of Directors of the Corporation, by written consent effective as of the 14th day of March, 2000, duly adopted the following resolutions:

RESOLVED: That, pursuant to Section 253 of the Delaware General Corporation Law, the Corporation is hereby authorized to merge ProScript, Inc., a Delaware corporation which is a wholly owned subsidiary of the Corporation, into the Corporation;

RESOLVED: That the President and Secretary of the Corporation be and each hereby is, authorized to execute a Certificate of Ownership and Merger with respect to the merger of ProScript, Inc. into the Corporation, cause the same to be filed with the Secretary of State of Delaware and take all such other actions and to execute all such other instruments and agreements as they or any of them may deem appropriate to effect such merger;

RESOLVED: That the merger of ProScript, Inc. into the Corporation shall be effective upon the filing of a Certificate of Ownership and Merger with the Secretary of State of Delaware.

STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 12:00 PM 03/16/2000
001133461 – 2296418

IN WITNESS WHEREOF, the Corporation has caused this Certificate to be signed by its authorized officer this 15th day of March, 2000.

LEUKOSITE, INC.

By: _____

Title: President
John Maraganore

UNITED STATES PATENT AND TRADEMARK OFFICE

MARCH 08, 2002

PTAS

Chief Information Officer
Washington, DC 20231
www.uspto.gov

HALE AND DORR LLP
JANICE M. KLUNDER, PH.D.
60 STATE STREET
BOSTON, MASSACHUSETTS 02109

101951033A

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PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

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REEL/FRAME: 012454/0218
NUMBER OF PAGES: 6

BRIEF: MERGER (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
LEUKOSITE, INC., A CORP. OF DELAWARE

DOC DATE: 03/15/2000

ASSIGNEE:
MILLENNIUM PHARMACEUTICALS, INC.,
A CORP. OF DELAWARE
75 SIDNEY STREET
CAMBRIDGE, MASSACHUSETTS 02139

SERIAL NUMBER: 08549318
PATENT NUMBER: 5780454

FILING DATE: 10/27/1995
ISSUE DATE: 07/14/1998

ALLYSON PURNELL, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

HALE & DORR DOCKETING
RE: 103576.135 US 3
Action Date:
Action to be Taken:
Docketed by: AM On: 3/14/02

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- [ ] Other

U.S. Government
(For Use ONLY by U.S. Government Agencies)
- [ ] Departmental File
- [ ] Secret File

Conveying Party(ies)

- [ ] Mark if additional names of conveying parties attached

Name (line 1): LeukoSite, Inc.
Execution Date (Month Day Year): 03 15 00

Name (line 2): a Delaware Corporation

Second Party
Name (line 1):
Execution Date (Month Day Year):

Name (line 2):

Receiving Party

- [ ] Mark if additional names of receiving parties attached

Name (line 1): Millennium Pharmaceuticals, Inc.

Name (line 2): a Delaware Corporation

Address (line 1): 75 Sidney Street

Address (line 2):

Address (line 3): Cambridge (City) MA/USA (State/Country) 02139 (Zip Code)

- [ ] If document to be recorded is an assignment and the receiving party is not domiciled in the United States, an appointment of a domestic representative is attached. *(Designation must be a separate document from Assignment.)*

Domestic Representative Name and Address

Enter for the first Receiving Party only.

Name:

Address (line 1):

Address (line 2):

Address (line 3):

Address (line 4):

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Expires 06/30/99
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U.S. Department of Commerce
Patent and Trademark Office
PATENT

Correspondent Name and Address

Area Code and Telephone Number: 617/526-6771

Name: Janice M. Klunder, Ph.D.

Address (line 1): Hale and Dorr LLP

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Address (line 4): Boston, MA 02109

Pages

Enter the total number of pages of the attached conveyance document including any attachments. # 3

Application Number(s) or Patent Number(s)

☐ Mark if additional numbers attached

Enter either the Patent Application Number or the Patent Number (DO NOT ENTER BOTH numbers for the same property).

Patent Application Number(s)			Patent Number(s)		
			5780454		

If this document is being filed together with a new Patent Application, enter the date the patent application was signed by the first named executing inventor. Month Day Year

Patent Cooperation Treaty (PCT)

Enter PCT application number only if a U.S. Application Number has not been assigned.

PCT PCT PCT

PCT PCT PCT

Number of Properties

Enter the total number of properties involved. # 1

Fee Amount

Fee Amount for Properties Listed (37 CFR 3.41): $ 40.00

Method of Payment: Enclosed ☐ Deposit Account ☑

Deposit Account

(Enter for payment by deposit account or if additional fees can be charged to the account.)

Deposit Account Number: # 08-0219

Authorization to charge additional fees: Yes ☑ No ☐

Statement and Signature

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document. Charges to deposit account are authorized, as indicated herein.

Janice M. Klunder, Ph.D., Reg. No. 41,121	Janice M. Klunder	11/27/01
Name of Person Signing	Signature	Date

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☐ Mark if additional names of conveying parties attached

Enter additional Conveying Parties

Name (line 1) _____ Execution Date Month Day Year _____

Name (line 2) _____

Name (line 1) _____ Execution Date Month Day Year _____

Name (line 2) _____

Name (line 1) _____ Execution Date Month Day Year _____

Name (line 2) _____

Receiving Party(ies)

☐ Mark if additional names of receiving parties attached

Enter additional Receiving Party(ies)

Name (line 1) _____ ☐ If document to be recorded is an assignment and the receiving party is not domiciled in the United States, an appointment of a domestic representative is attached. *(Designation must be a separate document from Assignment.)*

Name (line 2) _____

Address (line 1) _____

Address (line 2) _____

Address (line 3) _____ City _____ State/Country _____ Zip Code

Name (line 1) _____ ☐ If document to be recorded is an assignment and the receiving party is not domiciled in the United States, an appointment of a domestic representative is attached. *(Designation must be a separate document from Assignment.)*

Name (line 2) _____

Address (line 1) _____

Address (line 2) _____

Address (line 3) _____ City _____ State/Country _____ Zip Code

Application Number(s) or Patent Number(s)

☐ Mark if additional numbers attached

Enter either the Patent Application Number or the Patent Number (DO NOT ENTER BOTH numbers for the same property).

Patent Application Number(s)			Patent Number(s)		

State of Delaware

Office of the Secretary of State

I, EDWARD J. FREEL, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF OWNERSHIP, WHICH MERGES:

"LEUKOSITE, INC.", A DELAWARE CORPORATION,

WITH AND INTO "MILLENNIUM PHARMACEUTICALS, INC." UNDER THE NAME OF "MILLENNIUM PHARMACEUTICALS, INC.", A CORPORATION ORGANIZED AND EXISTING UNDER THE LAWS OF THE STATE OF DELAWARE, AS RECEIVED AND FILED IN THIS OFFICE THE SIXTEENTH DAY OF MARCH, A.D. 2000, AT 5:30 O'CLOCK P.M.

Edward J. Freel, Secretary of State

2296418 8100M

001315321

0512437

AUTHENTICATION:

06-21-00

DATE:

CERTIFICATE OF OWNERSHIP AND MERGER

MERGING

LeukoSite, Inc.
(a Delaware corporation)

INTO

Millennium Pharmaceuticals, Inc.
(a Delaware corporation)

Millennium Pharmaceuticals, Inc., a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware (the "Corporation"), does hereby certify:

FIRST: That the Corporation was incorporated on the 13th day of January, 1993 pursuant to the General Corporation Law of the State of Delaware.

SECOND: That the Corporation owns all of the outstanding shares of each class of the stock of LeukoSite, Inc., a corporation incorporated on the 1st day of May, 1992 pursuant to the General Corporation Law of the State of Delaware.

THIRD: That the Executive Committee of the Board of Directors of the Corporation, by written consent effective as of the 13th day of March, 2000, duly adopted the following resolutions:

RESOLVED: That, pursuant to Section 253 of the Delaware General Corporation Law, the Corporation is hereby authorized to merge LeukoSite, Inc., a Delaware corporation which is a wholly owned subsidiary of the Corporation, into the Corporation;

RESOLVED: That the President and Secretary of the Corporation be and each hereby is, authorized to execute a Certificate of Ownership and Merger with respect to the merger of LeukoSite, Inc. into the Corporation, cause the same to be filed with the Secretary of State of Delaware and take all such other actions and to execute all such other instruments and agreements as they or any of them may deem appropriate to effect such merger;

RESOLVED: That the merger of LeukoSite, Inc. into the Corporation shall be effective upon the filing of a Certificate of Ownership and Merger with the Secretary of State of Delaware.

STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 05:30 PM 03/16/2000
001135148 – 2322355

IN WITNESS WHEREOF, the Corporation has caused this Certificate to be signed by its authorized officer this 15th day of March, 2000.

MILLENNIUM PHARMACEUTICALS, INC.

By: _____

Title: Secretary
Jack Douglas

B

Millennium Pharmaceuticals, Inc.
75 Sidney Street
Cambridge, MA 02139
Tel 617 679 7000
www.mlnm.com

August 3, 2001

This letter is to confirm that in accordance with a Written Action of the Executive Committee of the Board of Directors of Millennium Pharmaceuticals, Inc. resolved June 21, 2001 (copy attached), Scott A. Brown, Associate General Counsel and Chief Patent Counsel of Millennium Pharmaceuticals, Inc. is authorized to sign Powers of Attorney, Assignments and all other documents that are required to be executed by a duly authorized representative of the company in connection with Millennium Pharmaceuticals, Inc.'s domestic and foreign intellectual property.

With best regards,

John B. Douglas III
Senior Vice President, General Counsel and Secretary

/djc
Attachment

MILLENNIUM PHARMACEUTICALS, INC.

Written Action of the Executive Committee of the Board of Directors

June 21, 2001

The undersigned, being all of the members of the Executive Committee of the Board of Directors of Millennium Pharmaceuticals, Inc., a Delaware corporation (the "Company"), and acting in accordance with Section 141(f) of the Delaware General Corporation Law and the By-laws of the Company, hereby adopt the following resolutions in lieu of a meeting of the Executive Committee:

Signatories

RESOLVED: That the first resolution under the subtitle "signatories" approved by the Board of Directors on September 28, 2000 is hereby terminated.

FURTHER RESOLVED: That, except as the Board of Directors may generally or in particular cases authorize the execution thereof in some other manner, the Chief Executive Officer, the Chief Financial Officer and the General Counsel of the Company and their respective successors, are, and each of them acting singly is, hereby authorized to designate from time to time persons authorized as signatories for and on behalf of the Company with the authority to execute documents and instruments that are within the ordinary course of the Company's business.

FURTHER RESOLVED: That, except as the Board of Directors may generally or in particular cases authorize the execution thereof in some other manner, the General Counsel and the Chief Patent Counsel and their respective successors, be, and each of them acting singly hereby is, authorized as a signatory for and on behalf of the Company with the authority to execute the following kinds of documents and instruments that are within the ordinary course of the Company's business:

(a) documents and instruments that are required to be executed by a duly authorized representative of the Company in connection with the Company's U.S. and foreign intellectual property, including patent, trademark and copyright prosecution before the U.S. and foreign patent, trademark and copyright offices, including, but not limited to Requests for Small Entity Status and PCT Applications; and

(b) powers of attorney and consulting agreements related to the foregoing.

EXECTUED as of the date first written above.

Mark J. Levin

A. Grant Heidrich, III

US005780454A

United States Patent [19]

Adams et al.

[11] Patent Number: 5,780,454

[45] Date of Patent: Jul. 14, 1998

[54] BORONIC ESTER AND ACID COMPOUNDS

[75] Inventors: Julian Adams, Brookline; Yu-Ting Ma, Needham; Ross Stein, Sudbury; Matthew Baevsky, Jamaica Plains; Louis Grenier; Louis Plamondon, both of Belmont, all of Mass.

[73] Assignee: ProScript, Inc., Cambridge, Mass.

[21] Appl. No.: 549,318

[22] Filed: Oct. 27, 1995

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 442,581, May 16, 1995, which is a continuation-in-part of Ser. No. 330,525, Oct. 28, 1994, abandoned.

[51] Int. Cl.[6] C07F 5/02; C07F 5/04; A61K 31/69

[52] U.S. Cl. 514/64; 544/229

[58] Field of Search 544/229; 514/64

[56] References Cited

U.S. PATENT DOCUMENTS

5,550,262	8/1996	Iqbal et al. .
5,614,649	3/1997	Iqbal et al. .

FOREIGN PATENT DOCUMENTS

0 471 651	2/1992	European Pat. Off. .
WO 92/07869	5/1992	WIPO .
WO 93/21213	10/1993	WIPO .
WO 93/21214	10/1993	WIPO .
WO 94/21668	9/1994	WIPO .
95/09858	4/1995	WIPO .

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Bachovin, W.W., et al., "Nitrogen-15 NMR Spectroscopy of the Catalytic-Triad Histidine of a Serine Protease in peptide Boronic Acid Inhibitor Complexes," *Biochemistry* 27:7689-7697 (1988).

Berry, S.C., et al., "Interaction of Peptide Boronic Acids With Elastase: Circular Dichroism Studies," *Proteins: Structure, Function, and Genetics* 4:205-210 (1988).

Kettner, C.A., et al., "Kinetic Properties of the Binidng of α-Lytic Protease to Peptide Boronic Acids," *Biochemistry* 27:7682-7688 (1988).

Kinder, D.H., and Katzenellenbogen, J.A., "Acylamino Boronic Acids and Difluoroborane Analogues of Amino Acids: Potent Inhibitors of Chymotrypsin and Elastase," *J. Med. Chem.* 28:1917-1925 (1985).

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Matteson, D.S., et al., "(R)-1-Acetamido-2-phenylethaneboronic Acid. A Specific Transition-State Analogue for Chymotrypsin," *J. Am. Chem. Soc.* 103:5241-5242 (1981).

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Tsilikounas, E., et al., "Identification of Serine and Histidine Adducts in Complexes of Trypsin and Trypsinogen with peptide and Nonpeptide Boronic Acid Inhibitors by ^{1}H NMR Spectroscopy," *Biochemistry* 31:12839-12846 (1992).

Veale, C.A., et al., "Nonpeptidic Inhibitors of Human Leukocyte Elastase. 5. Design, Synthesis, and X-ray Crystallography of a Series of Orally Active 5-Aminpyrimidin-6-one-Containing Trifluoromethyl Ketones," *J. Med. Chem.* 38(1):98-108 (Jan. 6, 1995).

Primary Examiner—Robert W. Ramsuer

Attorney, Agent, or Firm—Sterne, Kessler, Goldstein & Fox, PLLC

[57] ABSTRACT

Disclosed herein is a method for reducing the rate of degradation of proteins in an animal comprising contacting cells of the animal with certain boronic ester and acid compounds. Also disclosed herein are novel boronic ester and acid compounds, their synthesis and uses.

22 Claims, 3 Drawing Sheets

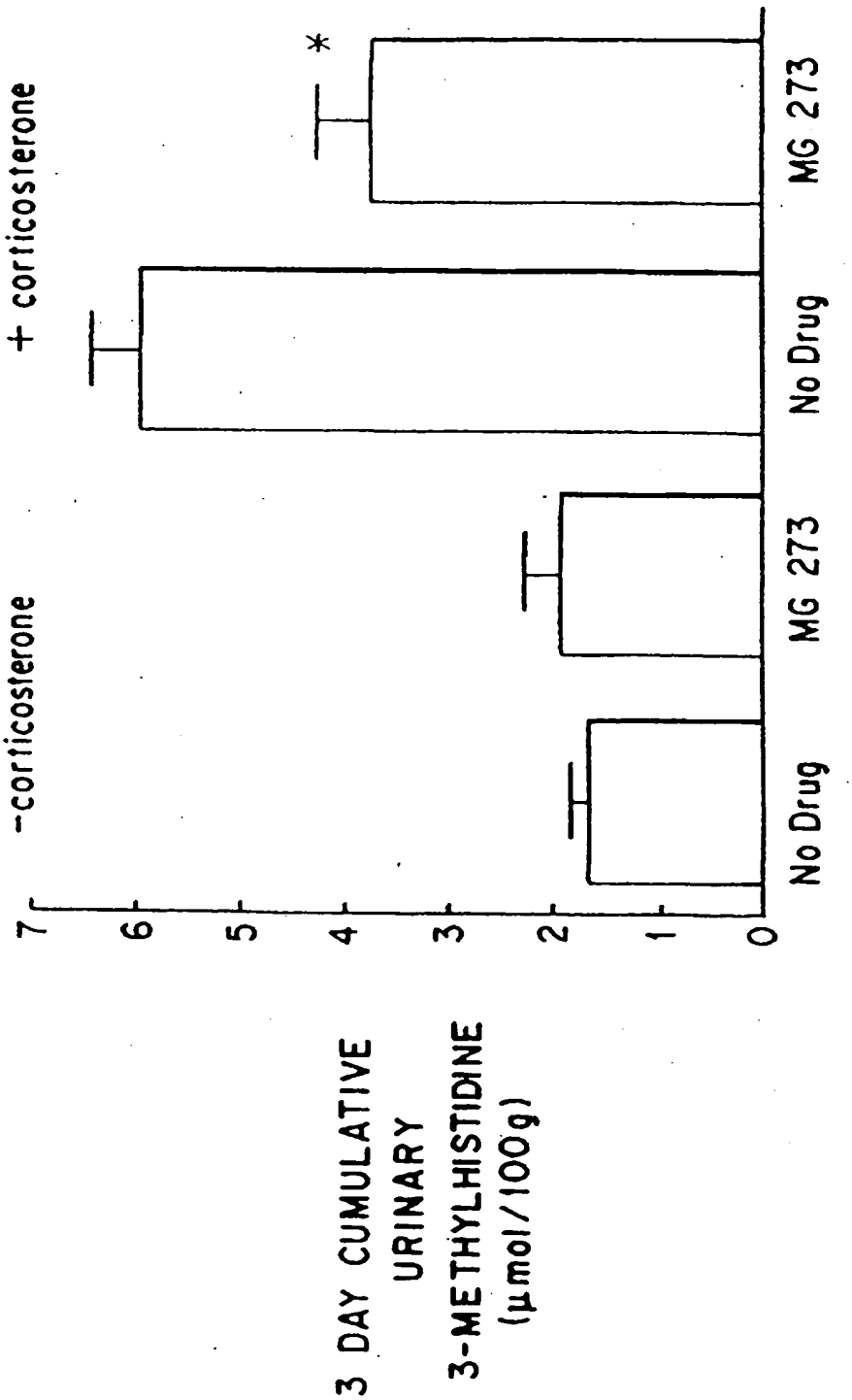

3 DAY CUMULATIVE URINARY 3-METHYLHISTIDINE (μmol/100g)
0
1
2
3
4
5
6
7
-corticosterone
+ corticosterone
No Drug
MG 273
No Drug
MG 273
*
*p<0.05

FIG. 1

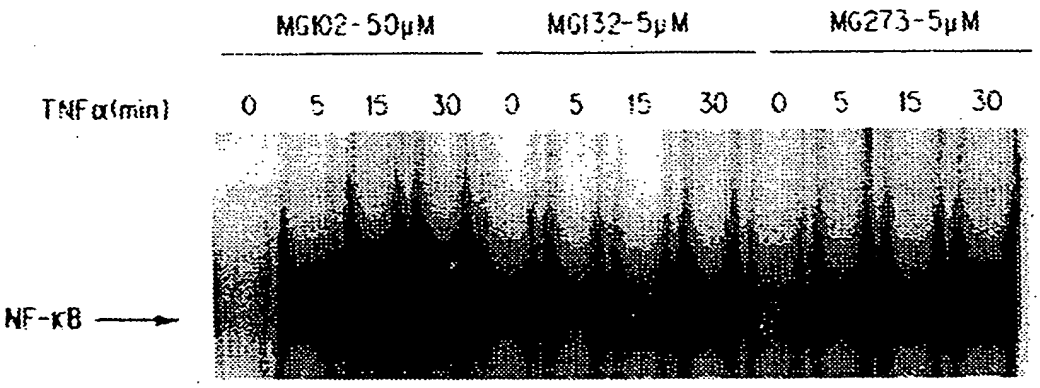

MG102-50μM
MG132-5μM
MG273-5μM
TNFα(min)
0 5 15 30 0 5 15 30 0 5 15 30
NF-κB
MG63 CELLS

FIG. 2

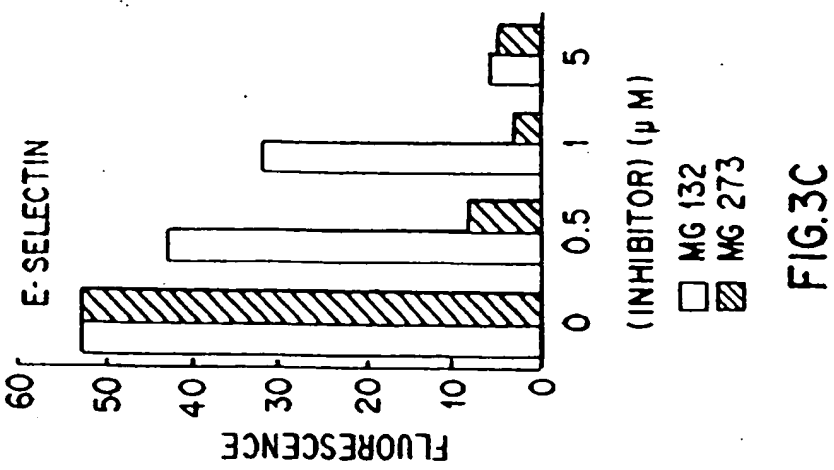


FIG.3C

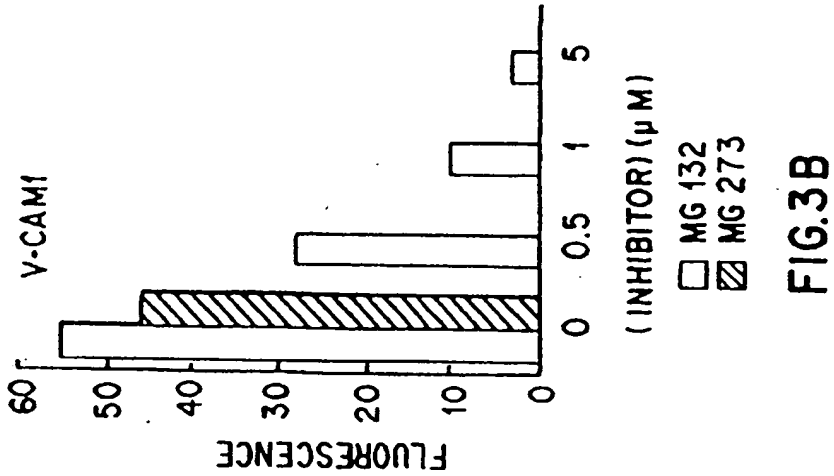


FIG.3B

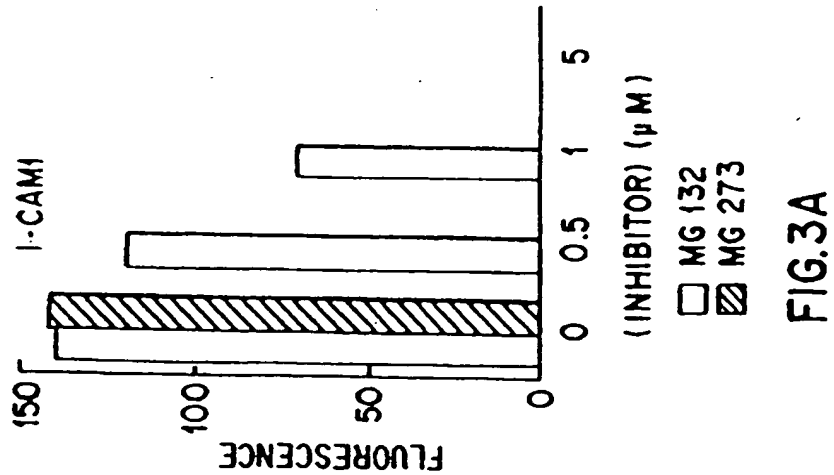


FIG.3A

BORONIC ESTER AND ACID COMPOUNDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Ser. No. 08/442,581, filed May 16, 1995, which is a continuation-in-part of U.S. application No. 08/330,525, filed Oct. 28, 1994, now abandoned, the contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to boronic ester and acid compounds, their synthesis and uses.

2. Description of Related Art

The synthesis of N-terminal peptidyl boronic ester and acid compounds, in general and of specific compounds, has been described previously (Shenvi et al. U.S. Pat. No. 4,499,082 issued Feb. 12, 1985; Shenvi et al. U.S. Pat. No. 4,537,773 issued Aug. 27, 1985; Siman et al. WO 91/13904 published Sep. 19, 1991; Kettner et al., *J Biol. Chem.* 259(24):15106–15114 (1984)). These compounds have been shown to be inhibitors of certain proteolytic enzymes (Shenvi et al. U.S. Pat. No. 4,499,082 issued Feb. 12, 1985; Shenvi et al. U.S. Pat. No. 4,537,773; Siman et al. WO 91/13904 published Sep. 19, 1991; Kettner et al., *J Biol. Chem.* 259(24):15106–151 14 (1984)). A class of N-terminal tri-peptide boronic ester and acid compounds has been shown to inhibit the growth of cancer cells (Kinder et al. U.S. Pat. No. 5,106,948 issued Apr. 21, 1992). A broad class of N-terminal tri-peptide boronic ester and acid compounds and analogs thereof has been shown to inhibit renin (Kleeman et al. U.S. Pat. No. 5,169,841 issued Dec. 8, 1992).

In the cell, there is a soluble proteolytic pathway that requires ATP and involves covalent conjugation of the cellular proteins with the small polypeptide ubiquitin ("Ub") (Hershko et al., *A. Rev. Biochem.* 61:761–807 (1992); Rechsteiner et al., *A. Rev. Cell. Biol.* 3:1–30 (1987)). Thereafter, the conjugated proteins are hydrolyzed by a 26S proteolytic complex containing a 20S degradative particle called the proteasome (Goldberg, *Eur. J Biochem.* 203:9–23 (1992); Goldberg et al., *Nature* 357:375–379 (1992)). This multi-component system is known to catalyze the selective degradation of highly abnormal proteins and short-lived regulatory proteins.

The 20S proteasome is composed of about 15 distinct 20–30 kDa subunits. It contains three different peptidase activities that cleave specifically on the carboxyl side of the hydrophobic, basic, and acidic amino acids (Goldberg et al., *Nature* 357:375–379 (1992); Goldberg, *Eur. J. Biochem.* 203:9–23 (1992); Orlowski, *Biochemistry* 29:10289 (1990); Rivett et al., *Archs. Biochem. Biophys.* 218:1 (1989); Rivett et al., *J. Biol. Chem.* 264:12,215–12,219 (1989); Tanaka et al., *New Biol.* 4:1–11 (1992)). These peptidase activities are referred to as the chymotrypsin-like activity, the trypsin-like activity, and the peptidylglutamyl hydrolyzing activity, respectively.

Various inhibitors of the peptidase activities of the proteasome have been reported (Dick et al., *Biochemistry* 30:2725–2734 (1991); Goldberg et al., *Nature* 357:375–379 (1992); Goldberg, *Eur. J Biochem.* 203:9–23 (1992); Orlowski, *Biochemistry* 29:10289 (1990); Rivett et al., *Archs. Biochem. Biophys.* 218:1 (1989); Rivett et al., *J. Biol. Chem.* 264:12,215–12,219 (1989); Tanaka et al., *New Biol.* 4:1–11 (1992); Murakami et al., *Proc. Natl. Acad. Sci. U.S.A.* 83:7588–7592 (1986); Li et al., *Biochemistry* 30:9709–9715 (1991); Goldberg, *Eur. J. Biochem.* 203:9–23 (1992); Aoyagi et al., *Proteases and Biological Control.* Cold Spring Harbor Laboratory Press (1975), pp. 429–454.

Stein et al., U.S. patent application Ser. No. 08/212,909 filed Mar. 15, 1994, describe the use of peptide aldehydes to 1) reduce the rate of loss of muscle mass in an animal by contacting cells of the muscle with a peptide aldehyde proteasome inhibitor, 2) reduce the rate of intracellular protein breakdown in an animal by contacting cells of the animal with a peptide aldehyde proteasome inhibitor, and 3) reduce the rate of degradation of p53 protein in an animal by administering to the animal a peptide aldehyde proteasome inhibitor.

Palombella et al., PCT application Ser. No. PCT/US95/03315, filed Mar. 17, 1995, describe the use of peptide aldehydes to reduce the cellular content and activity of NF-κB in an animal by contacting cells of the animal with a peptide aldehyde inhibitor of proteasome function or ubiquitin conjugation.

The transcription factor NF-κB and other members of the rel family of protein complexes play a central role in the regulation of a remarkably diverse set of genes involved in the immune and inflammatory responses (Grilli et al., *International Review of Cytology* 143:1–62 (1993)). NF-κB exists in an inactive form in the cytoplasm complexed with an inhibitor protein, IκB. In order for the NF-κB to become active and perform its function, it must enter the cell nucleus. It cannot do this, however, until the IκB portion of the complex is removed, a process referred to by those skilled in the art as the activation of, or processing of, NF-κB. In some diseases, the normal performance of its function by the NF-κB can be detrimental to the health of the patient. For example, NF-κB is essential for the expression of the human immunodeficiency virus (HIV). Accordingly, a process that would prevent the activation of the NF-κB in patients suffering from such diseases could be therapeutically beneficial.

Goldberg and Rock, WO 94/17816, filed Jan. 27, 1994, describe the use of proteasome inhibitors to inhibit MHC-I antigen presentation. The ubiquitination/proteolysis pathway is shown to be involved in the processing of internalized cellular or viral antigens into antigenic peptides that bind to MHC-I molecules on an antigen presenting cell. Accordingly, inhibitors of this pathway would be useful for the treatment of diseases that result from undesired response to antigen presentation, including autoimmune diseases and transplant rejection.

Cyclins are proteins that are involved in cell cycle control in eukaryotes. Cyclins presumably act by regulating the activity of protein kinases, and their programmed degradation at specific stages of the cell cycle is required for the transition from one stage to the next. Experiments utilizing modified ubiquitin (Glotzer et al., *Nature* 349:132–138 (1991); Hershko et al., *J. Biol. Chem.* 266:376 (1991)) have established that the ubiquitination/proteolysis pathway is involved in cyclin degradation. Accordingly, compounds that inhibit this pathway would cause cell cycle arrest and would be useful in the treatment of cancer, psoriasis, restenosis, and other cell proliferative diseases.

SUMMARY OF THE INVENTION

The present invention provides previously unknown peptidyl boronic acid ester and acid compounds. The present invention also provides methods of using amino acid or

peptidyl boronic ester and acid compounds, in general, as inhibitors of proteasome function.

In a first embodiment, the present invention provides novel boronic acid and ester compounds having formula (1a) or (2a), as set forth below.

An additional aspect of the present invention is related to the discovery that amino acid and peptidyl boronic acids and esters, in general, are potent and highly selective proteasome inhibitors and can be employed to inhibit proteasome function. Inhibition of proteasome function has a number of practical therapeutic and prophylactic applications.

In a second embodiment, the present invention provides a method for reducing the rate of muscle protein degradation in a cell comprising contacting said cell with a proteasome inhibitor having formula (1b) or (2b) as defined below. This aspect of the present invention finds practical utility in inhibiting (reducing or preventing) the accelerated breakdown of muscle proteins that accompanies various physiological and pathological states and is responsible to a large extent for the loss of muscle mass (atrophy) that follows nerve injury, fasting, fever, acidosis, and certain endocrinopathies.

In a third embodiment, the present invention provides a method for reducing the activity of NF-κB in a cell comprising contacting the cell with a proteasome inhibitor of the formula (1b) or (2b), as set forth below. The inhibitors employed in the practice of the present invention are capable of preventing this activation. Thus, blocking NF-κB activity is contemplated as possessing important practical application in various areas of medicine, e.g., inflammation, sepsis, AIDS, and the like.

In a fourth embodiment, the present invention provides a method of reducing the rate of degradation of p53 protein in a cell comprising administering to the cell a proteasome inhibitor of the formula (1b) or (2b), as set forth below.

In a fifth embodiment, the present invention provides a method for inhibiting cyclin degradation in a cell comprising contacting said cells with a proteasome inhibitor of the formula (1b) or (2b), as set forth below. Inhibiting cyclin degradation is contemplated as possessing important practical application in treating cell proliferative diseases, such as cancer, restenosis and psoriasis.

In a sixth embodiment, the present invention provides a method for inhibiting the growth of a cancer cell, comprising contacting said cell with a proteasome inhibitor of the formula (1a) or (2a), as set forth below.

In a seventh embodiment, the present invention provides a method for inhibiting antigen presentation in a cell comprising administering to the cell a proteasome inhibitor of the formula (1b) or (2b), as set forth below.

In an eighth embodiment, the present invention provides a method for inhibiting inducible NF-κB dependent cell adhesion in an animal comprising administering to said animal a proteasome inhibitor of the formula (1b) or (2b), as set forth below.

In a ninth embodiment, the present invention provides a method for inhibiting HIV replication in an animal comprising administering to said animal a proteasome inhibitor of the formula (1b) or (2b), as set forth below.

In a tenth embodiment, the present invention provides an approach for inhibiting cytolytic immune responses. The proteasome inhibitors of formula (1b) or (2b) can be used to inhibit the processing of internalized cellular or viral antigens into antigenic peptides that bind to MHC-I molecules in an animal, and are therefore useful for treating autoimmune diseases and preventing rejection of foreign tissues, such as transplanted organs or grafts.

In an eleventh embodiment, the present invention provides pharmaceutical compositions that comprise compounds of formula (1a), (1b), (2a) or (2b) in an amount effective to inhibit proteasome function in a mammal, and a pharmaceutically acceptable carrier or diluent.

BRIEF DESCRIPTION OF THE FIGURES

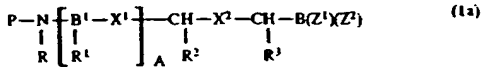
FIG. 1. Three day cumulative urinary 3-methylhistidine.

FIG. 2. NF-κB binding activity.

FIG. 3. Inhibition by MG-273.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

A first aspect of the present invention is directed to novel subsets of boronic acid and ester compounds having formula (1a) or (2a) below. Novel compounds of formula (1a) include the following:



(1a)

or a pharmaceutically acceptable salt thereof; wherein

P is hydrogen or an amino-group-protecting moiety as further defined herein;

B^1, at each occurrence, is independently one of N or CH;

X^1, at each occurrence, is independently one of —C(O)—NH—, —CH_2—NH—, —CH(OH)—CH_2—, —CH(OH)—CH(OH)—, —CH(OH)—CH_2—NH—, —CH=CH—, —C(O)CH_2—, —SO_2—NH—, —SO_2—CH_2— or —CH(OH)—CH_2—C(O)—NH—, provided that when B^1 is N, then the X^1 attached to said B^1 is —C(O)—NH—;

X^2 is one of —C(O)—NH—, —CH(OH)—CH_2—, —CH(OH)—CH(OH)—, —C(O)—CH_2—, —SO_2—NH—, —SO_2—CH_2— or —CH(OH)—CH_2—C(O)—NH—;

R is hydrogen or alkyl, or R forms together with the adjacent R^1, or when A is zero, forms together with the adjacent R^2, a nitrogen-containing mono-, bi- or tricyclic, saturated or partially saturated ring system having 4–14 ring members, that can be optionally substituted by one or two of keto, hydroxy, alkyl, aryl, aralkyl, alkoxy or aryloxy;

R^1, at each occurrence, is independently one of hydrogen, alkyl, cycloalkyl, aryl, a 5–10 membered saturated, partially unsaturated or aromatic heterocycle or —CH_2—R^5, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

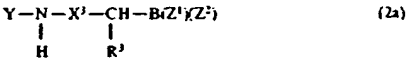
R^2 is one of hydrogen, alkyl, cycloalkyl, aryl, a 5–10 membered saturated, partially unsaturated or aromatic heterocycle or —CH—R^5, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

R^3 is one of hydrogen, alkyl, cycloalkyl, aryl, a 5–10 membered saturated, partially unsaturated or aromatic heterocycle or —CH_2—R^5, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

R^5, in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, a 5–10 membered saturated, partially unsaturated or aromatic heterocycle or —W—R^6, where W is a chalcogen and R^6 is alkyl, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

Z^1 and Z^2 are independently one of alkyl, hydroxy, alkoxy, or aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising carbon atoms, and optionally, a heteroatom or heteroatoms which can be N, S, or O; and A is 0, 1, or 2.

Other novel boronic acid and ester derivatives include compounds having a single amino acid side-chain. These compounds have the following formula:



(2a)

and pharmaceutically acceptable salts thereof; wherein

Y is one of R^8—C(O)—, R^8—SO_2—, R^8—NH—C(O)— or R^8—O—C(O)—, where R^8 is one of alkyl, aryl, alkaryl, aralkyl, any of which can be optionally substituted, or when Y is R^8—C(O)— or R^8—SO_2—, then R^8 can also be an optionally substituted 5–10 membered, saturated, partially unsaturated or aromatic heterocycle;

X^3 is a covalent bond or —C(O)—CH_2—;

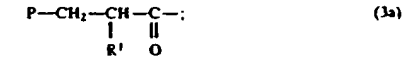
R^3 is one of hydrogen, alkyl, cycloalkyl, aryl, a 5–10 membered saturated, partially unsaturated or aromatic heterocycle or —CH_2—R^5, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

R^5, in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, a 5–10 membered saturated, partially unsaturated or aromatic heterocycle or —W—R^6, where W is a chalcogen and R^6 is alkyl, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted; and

Z^1 and Z^2 are independently alkyl, hydroxy, alkoxy, aryloxy, or together form a moiety derived from dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising carbon atoms, and optionally, a heteroatom or heteroatoms which can be N, S, or O;

provided that when Y is R^8—C(O)—, R^8 is other than phenyl, benzyl or C_1–C_3 alkyl.

Alternatively, the group Y in formula (2a) above, can be:



(3a)

P is one of R^7—C(O)—, R^7—SO_2—, R^7—NH—C(O)— or R^7—O—C(O)—;

R^7 is one of alkyl, aryl, alkaryl, aralkyl, any of which can be optionally substituted, or when Y is R^7—C(O)— or R^7—SO_2—, R^7 can also be an optionally substituted 5–10 membered saturated, partially unsaturated or aromatic heterocycle; and

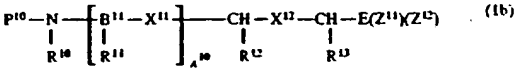
R^1 is defined above as for formula (1a).

Pharmaceutical compositions that comprise compounds of formula (1a) or (2a) in an amount effective to inhibit proteasome function in a mammal, and a pharmaceutically acceptable carrier or diluent are within the scope of the present invention.

A second aspect of the present invention lies in the discovery that boronic acid and ester derivatives of amino acids and peptides, in general, as well as isosteric variations thereof, inhibit proteasome function. Thus, the present invention also relates to the use of proteasome inhibitors having formula (1b) or (2b) for reducing the rate of proteasome dependent intracellular protein breakdown, such as reducing the rate of muscle protein degradation, reducing the rate of degradation of p53 protein, and inhibiting cyclin degradation, and for inhibiting the activity of NF-κB in a cell.

Finally, the present invention relates to the use of proteasome inhibitors having formula (1b) or (2b) for treating specific conditions in animals that are mediated or exacerbated, directly or indirectly, by proteasome functions. These conditions include inflammatory conditions, such as tissue rejection, organ rejection, arthritis, infection, dermatoses, inflammatory bowel disease, asthma, osteoporosis, osteoarthritis and autoimmune disease such as lupus and multiple sclerosis; cell proliferative diseases, such as cancer, psoriasis and restenosis; and accelerated muscle protein breakdown that accompanies various physiological and pathological states and is responsible to a large extent for the loss of muscle mass (atrophy) that follows nerve injury, fasting, fever, acidosis, and certain endocrinopathies.

Proteasome inhibitors of formula (1b) include:



(1b)

or a pharmaceutically acceptable salt thereof; wherein

P^{10} is hydrogen or an amino-group-protecting moiety;

B^{11} is independently one of N or CH;

X^{11}, at each occurrence, is independently one of —C(O)—NH—, —CH_2—NH—, —CH(OH)—CH_2—, —CH(OH)—CH(OH)—, —CH(OH)—CH_2—NH—, —CH=CH—, —C(O)—C_2—, —SO_2—NH—, —SO_2—CH_2— or —CH(OH)—CH_2—C(O)—NH—, provided that when B^{11} is N, then X^{11} is —C(O)—NH;

X^{12} is one of —C(O)—NH—, —CH(OH)—CH_2—, —CH(OH)—CH(OH)—, —C(O)—CH_2—, —SO_2—NH—, —SO_2—CH_2- or —CH(OH)—CH_2—C(O)—NH—;

R^{10} is hydrogen or alkyl, or R^{10} forms together with the adjacent R^{11}, or when A^{10} is zero, forms together with the adjacent R^{12}, a nitrogen-containing mono-, bi- or tri-cyclic, saturated or partially saturated ring system having 4–14 ring members, that can be optionally substituted by one or two of keto, hydroxy, alkyl, aryl, aralkyl, alkoxy or aryloxy;

R^{11}, at each occurrence, is independently one of hydrogen, alkyl, cycloalkyl, aryl, a 5–10 membered saturated, partially unsaturated or aromatic heterocycle or —CH_2—R^{15}, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

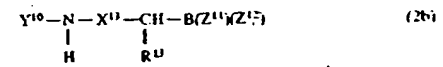
R^{12} and R^{13} are each independently one of hydrogen, alkyl, cycloalkyl, aryl, a 5–10 membered saturated, partially unsaturated or aromatic heterocycle or —CH_2—R^{15}, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted, where R^{15} is aryl, aralkyl, alkaryl, cycloalkyl, a 5–10 membered saturated, partially unsaturated or aromatic heterocycle, or -chalcogen-alkyl, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

Z^{11} and Z^{12} are independently alkyl, hydroxy, alkoxy, aryloxy, or Z^{11} and Z^{12} together form a moiety derived from a dihydroxy compound having at least two hydroxy groups separated by at least two connecting

atoms in a chain or ring, said chain or ring comprising carbon atoms, and optionally, a heteroatom or heteroatoms which can be N, S, or O; and

A^{10} is 0, 1, or 2

Proteasome inhibitors of formula (2b) include:



(2b)

or pharmaceutically acceptable salts thereof; wherein

Y^{10} is one of R^8—C(O)—, R^8—SO_2—, R^8—NH—C(O)— or R^8—O—C(O)—, where R^8 is one of alkyl, aryl, alkaryl, aralkyl, any of which can be optionally substituted, or when Y is R^8—C(O)— or R^8—SO_2—, then R^8 can also be an optionally substituted 5–10 membered, saturated, partially unsaturated or aromatic heterocycle;

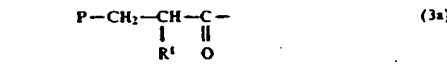
X^{13} is a covalent bond or —C(O)—CH_2—;

R^{13} is one of hydrogen, alkyl, cycloalkyl, aryl, a 5–10 membered saturated, partially unsaturated or aromatic heterocycle or —CH_2—R^{15}, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted;

R^{15}, in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, a 5–10 membered saturated, partially unsaturated or aromatic heterocycle or —W—R^{16}, where W is a chalcogen and R^{16} is alkyl, where the ring portion of any of said aryl, aralkyl, alkaryl or heterocycle can be optionally substituted; and

Z^{11} and Z^{12} are independently alkyl, hydroxy, alkoxy, aryloxy, or together form a moiety derived from a dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising carbon atoms, and optionally, a heteroatom or heteroatoms which can be N, S, or O.

Alternatively, the group Y in formula (2b) can be:



(3a)

P is one of R^7—C(O)—, R^7—SO_2—, R^7—NH—C(O)— or R^7—O—C(O)—;

R^7 is one of alkyl, aryl, alkaryl, aralkyl, any of which can be optionally substituted, or when Y is R^7—C(O)— or R^7—SO_2—, R^7 can also be an optionally substituted 5–10 membered saturated, partially unsaturated or aromatic 10 heterocycle; and

R^1 is as defined for formula (1a) above.

Preferred embodiments of the aforementioned methods of use employ compounds of formula (1a) and formula (2a) as defined above.

Pharmaceutical compositions comprising an effective amount of the proteasome inhibitors of formula (2a) or (2b), in combination with any conventional pharmaceutically acceptable carrier or diluent, are included in the present invention.

The term "amino-group-protecting moiety," as used herein, refers to terminal amino protecting groups that are typically employed in organic 20 synthesis, especially peptide synthesis. Any of the known categories of protecting groups can be employed, including acyl protecting groups, such as acetyl, and benzoyl; aromatic urethane protecting groups, such as benzyloxycarbonyl; and aliphatic urethane protecting groups, such as tert-butoxycarbonyl. See, for example, *The Peptides*, Gross and Meienhoffer, eds., Academic Press, New York (1981), Vol. 3, pp. 3–88; and Green, T. W. & Wuts, P. G. M., *Protective Groups in Organic Synthesis*, 2nd edition, John Wiley and Sons, Inc., New York (1991). Preferred protecting groups include aryl-, aralkyl-, heteroaryl- and heteroarylalkyl- carbonyl and sulfonyl moieties.

As used herein, the term "heterocycle" is intended to mean a stable 5- to 7- membered monocyclic or 7- to 10-membered bicyclic heterocyclic moieties that are either saturated or unsaturated, and which consist of carbon atoms and from 1 to 4 heteroatoms independently selected from the group consisting of N, O and S, wherein the nitrogen and sulfur heteroatoms can optionally be oxidized, the nitrogen can optionally be quaternized, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable formula. The heterocyclic rings described herein can be substituted on carbon or on a nitrogen atom if the resulting compound is stable. Examples of such heterocycles include, but are not limited to, pyridyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, benzothiophenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl or octahydroisoquinolinyl, azocinyl, triazinyl, 6H- 1,2,5-thiadiazinyl, 2H,6H- 1,5,2-dithiazinyl, thiophene(yl), thianthrenyl, furanyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathiinyl, 2H-pyrrolyl, pyrrole, imidazolyl, pyrazolyl, isothiazolyl, isoxazolyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, indolyl, 1H-indazolyl, purinyl, 4H-quinolizinyl, isoquinolinyl, quinolinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, pyrrolidinyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl or oxazolidinyl. Also included are fused ring and spiro compounds containing, for example, the above heterocycles.

The term "substituted", as used herein, means that one or more hydrogens of the designated moiety are replaced with a selection from the indicated group, provided that no atom's normal valency is exceeded, and that the substitution results in a stable compound. When a substituent is keto (i.e., =O), then 2 hydrogens attached to an atom of the moiety are replaced.

By "stable compound" or "stable formula" is meant herein a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture and formulation into an efficacious therapeutic agent.

The term "heteroaryl" as employed herein refers to groups having 5 to 14 ring atoms; 6, 10 or 14 π electrons shared in a cyclic array; and containing carbon atoms and 1, 2 or 3 oxygen, nitrogen or sulfur heteroatoms (where examples of heteroaryl groups are: thienyl, benzo[b]thienyl, naphtho[2,3-b]thienyl, thianthrenyl, furyl, pyranyl, isobenzofuranyl, benzoxazolyl, chromenyl, xanthenyl, phenoxathiinyl, 2H-pyrrolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, indolyl, indazolyl, purinyl, 4H-quinolizinyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl,

perimidinyl, phenanthrolinyl, phenazinyl, isothiazolyl, phenothiazinyl, isoxazolyl, furazanyl and phenoxazinyl groups).

The terms "substituted heteroaryl" or "optionally substituted heteroaryl," used in reference to R^1, refer to heteroaryl groups, as defined above, having one or more substituents selected from halogen, C_{1-6} alkyl, C_{1-6} alkoxy, carboxy, amino, C_{1-6} alkylamino and/or di(C_{1-6})alkylamino.

The term "aryl" as employed herein by itself or as part of another group refers to monocyclic or bicyclic aromatic groups containing from 6 to 12 carbons in the ring portion, preferably 6–10 carbons in the ring portion, such as phenyl, naphthyl or tetrahydronaphthyl.

The term "substituted aryl" as employed herein includes aryl groups, as defined above, that include one or two substituents on either the phenyl or naphthyl group selected from C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{1-6}alkyl(C_{3-8})cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, cyano, amino, C_{1-6} alkylamino, di(C_{1-6})alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6})alkoxy, trifluoromethyl, halogen, C_{1-6} alkoxy, C_{6-10} aryl(C_{1-6})alkoxy, hydroxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{6-10} aryl, C_{6-10} arylthio, C_{6-10} arylsulfinyl and/or C_{6-10} arylsulfonyl.

The term "alkyl" as employed herein includes both straight and branched chain radicals of up to 12 carbons, preferably 1–8 carbons, such as methyl, ethyl, propyl, isopropyl, butyl, t-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, undecyl and dodecyl.

The term "substituted alkyl" as employed herein includes alkyl groups as defined above that have one, two or three halo substituents, or one C_{1-6} alkyl(C_{6-10})aryl, halo(C_{6-10}) aryl, C_{3-8} cycloalkyl, C_{1-6} alkyl(C_{3-8})cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, hydroxy and/or carboxy.

The term "cycloalkyl" as employed herein includes saturated cyclic hydrocarbon groups containing 3 to 12 carbons, preferably 3 to 8 carbons, which include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclododecyl and cyclododecyl, any of which groups can be substituted with substituents such as halogen, C_{1-6} alkyl, alkoxy and/or hydroxy group.

The term "aralkyl" or "arylalkyl" as used herein by itself or as part of another group refers to C_{1-6} alkyl groups as discussed above having an aryl substituent, such as benzyl.

The term "halogen" or "halo" as used herein by itself or as part of another group refers to chlorine, bromine, fluorine or iodine with chlorine being preferred.

For medicinal use, the pharmaceutically acceptable acid and base addition salts, those salts in which the anion does not contribute significantly to toxicity or pharmacological activity of the organic cation, are preferred. Basic salts are formed by mixing a solution of a boronic acid (Z^1 and Z^2 are both OH) of the present invention with a solution of a pharmaceutically acceptable non-toxic base, such as, sodium hydroxide, potassium hydroxide, sodium bicarbonate, sodium carbonate, or an amino compound, such as choline hydroxide, Tris, bis-Tris, N-methylglucamine or arginine. Water-soluble salts are preferable. Thus, suitable salts include: alkaline metal salts (sodium, potassium etc.), alkaline earth metal salts (magnesium, calcium etc.), ammonium salts and salts of pharmaceutically acceptable amines (tetramethylammonium, triethylamine, methylamine, dimethylamine, cyclopentylamine, benzylamine, phenethylamine, piperidine monoethanolamine, diethanolamine, tris(hydroxymethyl)amine, lysine, arginine and N-methyl-D-glucamine).

The acid addition salts are obtained either by reaction of an organic base of formula (1a) or (2a) with an organic or inorganic acid, preferably by contact in solution, or by any of the standard methods detailed in the literature available to any practitioner skilled in the art. Examples of useful organic acids are carboxylic acids such as maleic acid, acetic acid, tartaric acid, propionic acid, fumaric acid, isethionic acid, succinic acid, cyclamic acid, pivalic acid and the like; useful inorganic acids are hydrohalide acids such as HCl, HBr, HI; sulfuric acid; phosphoric acid and the like. Preferred acids for forming acid addition salts include HCl and acetic acid.

The boronate esters of boronic acid compounds of the present invention are also preferred. These esters are formed by reacting the acid groups of the boronic acid with a hydroxy compound. Preferred hydroxy compounds are dihydroxy compounds, especially pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol or diethanolamine.

The P moiety of the proteasome inhibitor of formula (1a) is preferably one of R^7—C(O)—, R^7—SO_2—, R^7—NH—C(O)— or R^7—O—C(O)—, and R^7 is one of alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or heteroarylalkyl, the ring portion of any of which can be optionally substituted, or if Y is R^7—C(O)— or R^7—SO_2—, then R^7 can also be a saturated or partially unsaturated heterocycle.

More preferably, P is one of R^7—C(O)— or R^7—SO_2—, and R^7 is one of aryl, aralkyl, heteroaryl or heteroarylalkyl, any of which can be optionally substituted, or a saturated or partially unsaturated heterocycle.

Where R^7 is alkyl, it is preferably straight chained or branched alkyl of from 1 to 6 carbon atoms, more preferably 1–4 carbon atoms. Useful values include methyl, ethyl, propyl, butyl, isopropyl, isobutyl and tert-butyl, with methyl being most preferred. Additionally, where R^7 is alkaryl, aralkyl or heteroarylalkyl, the alkyl moiety thereof is also preferably one having from 1 to 4 carbon atoms, and most preferably 1 carbon atom.

Where R^7 is aryl, it is preferably aryl of from 5 to 10 carbon atoms, more preferably 6 to 10 carbon atoms. Where R^7 is heteroaryl, one or more of the carbon atoms of the aforementioned aryl is replaced by one to three of O, N, or S. The aryl and heteroaryl moieties may, if desired, be ring substituted. Useful ring substituents include one or two of hydroxy, nitro, trifluoromethyl, halogen, alkyl, alkoxy, cyano, C_{6-10} aryl, benzyl, carboxyalkoxy, amino, and guanidino. Preferred substituents include halogen, C_{1-6} alkyl, C_{1-6} alkoxy, phenyl and benzyl. Additionally, where R^7 is alkaryl, aralkyl or heteroarylalkyl, the above statements equally apply.

Useful R^7 aryl and aralkyl groups include phenyl, 4-tolyl, benzyl, phenethyl, naphthyl, and naphthylmethyl.

Preferred heteroaryl groups are quinolinyl, quinoxalinyl, pyridyl, pyrazinyl, furanyl or pyrrolyl. Useful values of R^7 heteroaryl include 8-quinolinyl, 2-quinoxalinyl, 2-pyrazinyl, 3-furanyl, 2-pyridyl, 3-pyridyl and 4-pyridyl.

Preferred saturated or partially saturated heterocycle moieties are 5-, 6-, 9- and 10- membered heterocycles having one, two or three ring heteroatoms selected from O, S or N. A useful value is N-morpholinyl.

Preferred cycloalkyl moieties include C_{3-10} cycloalkyl. Useful values include cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and cyclononyl.

Especially preferred values of P are 2-pyrazinecarbonyl, 8-quinolinesulfonyl and N-morpholinyl.

As noted above, A in formula (1a) and (1b) can be either 0, 1 or 2. Thus, when A is zero, the residue within the brackets is not present and the inhibitor is a dipeptide.

Similarly, where A is 1, the amino acid or isosteric residue within the brackets is present and the inhibitor is a tripeptide. Where A is 2, the inhibitor is a tetrapeptide. Most preferably, A is zero.

It is preferred that R^1, R^2, and R^3 in formula (1a) and (1b) are each independently one of hydrogen, C_{1-8} alkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, a 5-, 6-, 9- or 10- membered heteroaryl group, or $—CH_2—R^5$, and more preferably C_{1-8} alkyl or $—CH_2—R^5$ wherein R^1, R^2, R^3 and R^5 are optionally substituted. More preferably, R^1, R^2 and R^3 are each independently one of C_{1-4} alkyl, e.g., methyl, ethyl, propyl, butyl, isopropyl, isobutyl, sec-butyl and t-butyl, or $—CH_2—R^5$, where R^5 is one of cycloalkyl, aryl or heterocycle. R^5 is preferably one of C_{6-10} aryl, C_{6-10} ar(C_{1-6})alkyl, C_{1-6} alk(C_{6-10})aryl, C_{3-10} cycloalkyl, C_{1-8} alkoxy, C_{1-8} alkylthio or a 5-, 6-, 9- or 10- membered heteroaryl group.

The ring portion of any of said aryl, aralkyl, alkaryl or 5-, 6-, 9- or 10-membered heteroaryl groups of R^1, R^2, R^3 and R^5 can be optionally substituted by one or two substituents independently selected from the group consisting of C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{1-6} alkyl(C_{3-8})cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, cyano, amino, C_{1-6} alkylamino, di(C_{1-6})alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6})alkoxy, trifluoromethyl, halogen, C_{1-6} alkoxy, C_{6-10} aryl, C_{6-10} aryl(C_{1-6})alkyl, C_{6-10} aryl(C_{1-6})alkoxy, hydroxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{6-10} arylthio, C_{6-10} arylsulfinyl, C_{6-10} arylsulfonyl, C_{1-6} aryl, C_{1-6} alkyl(C_{6-10})aryl, and halo(C_{6-10})aryl.

It is more preferred that at least one of R^1 and R^2 is isobutyl or $—CH_2—R^5$, and most preferred that R^2 is $—CH_2—R^5$. It is preferred that R^5 is C_{6-10} aryl, a 5-, 6-, 9- or 10- membered heteroaryl group having one to three heteroatoms independently selected from O, N and S.

Most preferably, R^2 is isobutyl, 6-quinolinylmethyl, 3-indolylmethyl, 4-pyridylmethyl, 3-pyridylmethyl, 2-pyridylmethyl, benzyl, 1-naphthylmethyl, 2-naphthylmethyl, 4-fluorobenzyl, 4-benzyloxybenzyl, 4-(2'-pyridylmethoxy)benzyl or benzylnaphthylmethyl.

Preferably, R^3 is C_{1-12} alkyl, more preferably C_{1-6} alkyl, most preferably C_4 alkyl, such as isobutyl.

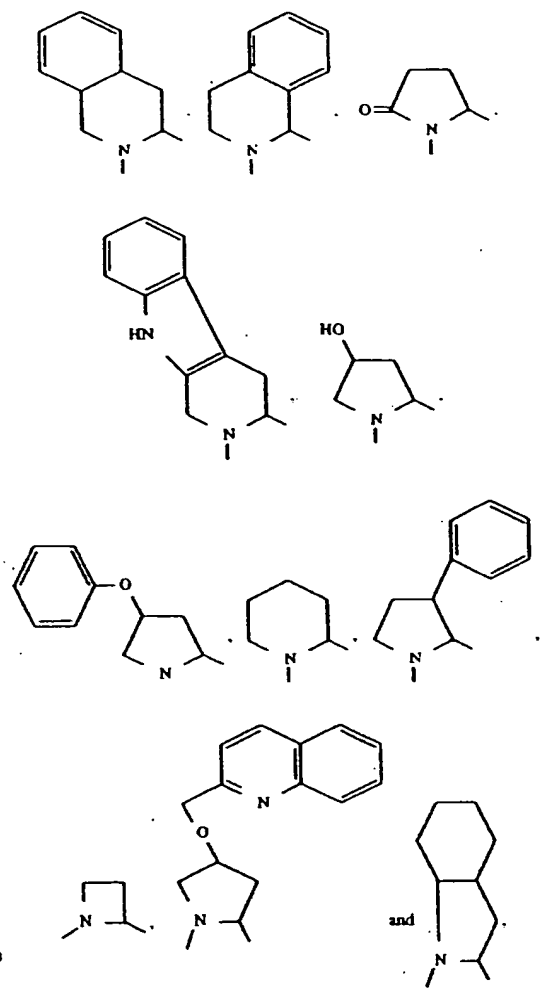
Where R^1, R^2 or R^3 is a substituted alkyl, it is preferably C_{1-4} alkyl substituted with at least one cycloalkyl group, preferably a C_{5-6} cycloalkyl group.

Where R^1, R^2, R^3, or R^5 is substituted aryl or substituted heterocycle, it is preferably substituted with at least one C_{1-4} alkyl group.

Where R^1, R^2, R^3 or R^5 is cycloalkyl, it is preferably C_{5-6} cycloalkyl, e.g., cyclopentyl or cyclohexyl, and can be optionally substituted with at least one C_{6-10} aryl group or at least one alkyl group, preferably a C_{1-4} alkyl group.

Where R^5 is $—W—R^6$, W is a chalcogen, preferably oxygen or sulfur, more preferably sulfur; and R^6 is alkyl, preferably C_{1-4} alkyl, e.g., methyl, ethyl, propyl, butyl, or isomers thereof.

Preferred values of R include hydrogen or C_{1-8} alkyl, more preferably C_{1-4} alkyl. Useful values of R include methyl, ethyl, isopropyl, isobutyl and n-butyl. Additionally, R can form together with the adjacent R^1, or when A is zero, form together with the adjacent R^2, a nitrogen-containing mono-, bi- or tri-cyclic, saturated or partially saturated ring system having 4–14 ring members, and can be optionally substituted by one or two of keto, hydroxy, aryl, alkoxy or aryloxy. It is preferred that the ring system be chosen from one of:



The nitrogen in each of the above formulae is attached to P in formula (1a) and the open valence carbon is attached to either X^1 or X^2.

It is preferred that Z^1 and Z^2 are each independently one of C_{1-4} alkyl, hydroxy, C_{1-6} alkoxy, and C_{6-10} aryloxy; or together Z^1 and Z^2 preferably form a moiety derived from a dihydroxy compound selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol or diethanolamine, or other equivalents apparent to those skilled in the art. Useful values include methyl, ethyl, propyl and n-butyl. Most preferably, Z^1 and Z^2 are hydroxy.

A preferred embodiment of the invention is directed to a subgenus of compounds having formula (1a) above, where P is $R^7—C(O)—$ or $R^7—SO_2—$, and R^7 is one of quinolinyl, quinoxalinyl, pyridyl, pyrazinyl, furanyl or pyrrolyl, and when P is $R^7—C(O)—$, R^7 can also be N-morpholinyl.

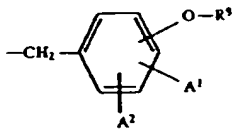
A preferred group of compounds of this embodiment are compounds of formula (1a) wherein P is one of quinolinecarbonyl, pyridinecarbonyl, quinolinesulfonyl, quinoxalinecarbonyl, quinoxalinesulfonyl, pyrazinecarbonyl, pyrazinesulfonyl, furancarbonyl, furansulfonyl or N-morpholinylcarbonyl; A is zero; X^2 is $—C(O)—NH—$; R is hydrogen or C_{1-8} alkyl; R^2 and R^3 are each

independently one of hydrogen, C_{1-6} alkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, C_{6-10} ar(C_{1-6})alkyl, pyridylmethyl, or quinolinylmethyl; and Z^1 and Z^2 are both hydroxy, C_{1-6} alkoxy, or C_{6-10} aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol or diethanolamine.

Even more preferred are those compounds wherein: P is 8-quinolinecarbonyl, 8-quinolinesulfonyl, 2-quinoxalinecarbonyl, 2-quinoxalinesulfonyl, 2-pyrazinecarbonyl, 2-pyrazinesulfonyl, 3-pyridinecarbonyl, 3-pyridinesulfonyl, 3-furancarbonyl, 3-furansulfonyl or N-morpholinecarbonyl; R is hydrogen; R^3 is isobutyl; R^2 is isobutyl, 1-naphthylmethyl, 2-naphthylmethyl, 3-pyridylmethyl, 2-pyridylmethyl 6-quinolinylmethyl, 3-indolylmethyl, benzyl, 4-fluorobenzyl, 4-hydroxybenzyl, 4-(2'-pyridylmethoxy) benzyl, 4-(benzyloxy)benzyl, benzylnaphthylmethyl or phenethyl; and Z^1 and Z^2 are both hydroxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol or diethanolamine.

Another preferred embodiment of the present invention is directed to compounds of formula (1a) where A is zero. These compounds possess unexpectedly high potency and selectivity as inhibitors of proteasome function.

A third preferred subgenus of compounds are compounds of formula (1a) where one of R^1, R^2 or R^3 corresponds to an amino acid side-chain corresponding to tyrosine or an O-substituted tyrosine derivative, formed by reacting the hydroxyl group of the tyrosine side-chain with a compound having a reactive functional group. This subgenus includes compounds having the formula (1a), wherein at least one R^1, R^2 or R^3 is:



where R^9 is one of hydrogen, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or heteroarylalkyl, wherein the alkyl is optionally substituted with one of C_{1-6} alkyl, halogen, monohalo (C_{1-6}) alkyl, and trifluoromethyl; and wherein said cycloalkyl, aryl, aralkyl, heteroaryl and heteroarylalkyl groups can be optionally substituted with one or two of C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{1-6} alkyl(C_{3-8})cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, cyano, amino, C_{1-6} alkylamino, di(C_{1-6})alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6}) alkoxy, trifluoromethyl, halogen, C_{1-6} alkoxy, C_{6-10} ryl, C_{6-10} aryl (C_{1-6})alkyl, C_{6-10} aryl(C_{6-10})alkoxy, hydroxy C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{6-10} arylthio, C_{6-10} arylsulfinyl, C_{6-10} aryl, C_{1-6}alkyl(C_{6-10})aryl, and halo(C_{6-10})aryl; and A^1 and A^2 are independently one of hydrogen, C_{1-6} alkyl, halogen, monohalo(C_{1-6})alkyl, or trifluoromethyl.

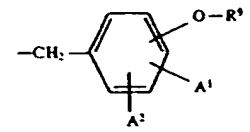
The group —O—R^9 is in either the ortho- or para-position, with para- being preferred. The groups A^1 and A^2 can be at any remaining positions on the phenyl ring.

It is preferred that R^9 is one of C_{1-8} alkyl, C_{3-10} cycloalkyl, C_{6-10} aryl, C_{6-10} ar (C_{1-6})alkyl, 5- to 10- membered heteroaryl or 5- to 10-membered heteroaryl(C_{1-6}) alkyl.

Useful values of R^9 include benzyl, phenethyl, pyridyl, pyridylmethyl, furanylmethyl pyrrolymethyl, pyrrolidylmethyl, oxazolylmethyl and imidazolylmethyl.

The ring portion of any of said aryl, aralkyl, alkaryl or 5-, 6-, 9- or 10-membered heteroaryl groups of R^1, R^2, R^3 and R^5 can be optionally substituted by one or two substituents independently selected from the group consisting of C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{1-6} alkyl(C_{3-8})cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, cyano, amino, C_{1-6} alkylamino, di(C_{1-6})alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6})alkoxy, trifluoromethyl, halogen, C_{1-6} alkoxy, C_{6-10} aryl, C_{6-10} aryl(C_{1-6})alkyl, C_{6-10}aryl(C_{1-6})alkoxy, hydroxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{6-10} arylthio, C_{6-10} arylsulfinyl, C_{6-10} arylsulfonyl, C_{6-10} aryl, C_{1-6} alkyl(C_{6-10})aryl, and halo(C_{6-10})aryl.

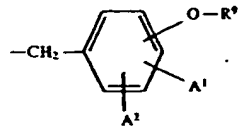
A preferred class of compounds of this embodiment are compounds of formula (1a) wherein: A is zero; P is one of R^7—C(O)—, R^7—SO_2—, R^7—NH—C(O)— or R^7—O—C(O)—; R^7 is one of quinolinyl, quinoxalinyl, pyridyl, pyrazinyl, furanyl or pyrrolyl, or when P is R^7—C(O)—, R^7 can also be N-morpholinyl; X^2 is —C(O)—NH—; R^3 is C_{1-6} alkyl, R^2 is:



where A^1 and A^2 are independently one of hydrogen, C_{1-6} alkyl, halogen, monohalo(C_{1-6})alkyl or trifluoromethyl; and R^9 is one of hydrogen, C_{1-8} alkyl, phenyl, benzyl, phenethyl or pyridylmethyl; and

Z^1 and Z^2 are both hydroxy, C_{1-6}alkoxy, or C_{6-10} aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol or diethanolamine.

Even more preferred are compounds of formula (1a) wherein: A is zero; P is 8-quinolinecarbonyl, 8-quinolinesulfonyl, 2-quinoxalinecarbonyl, 2-quinoxalinesulfonyl, 2-pyrazinecarbonyl, 2-pyrazinesulfonyl, 3-pyridinecarbonyl, 3-pyridinesulfonyl, 3-furancarbonyl, 3-furansulfonyl or N-morpholinecarbonyl; X^2 is —C(O)—NH—; R^3 is isobutyl; R^2 is:



where A^1 and A^2 are independently one of hydrogen, methyl, ethyl, chloro, fluoro, or trifluoromethyl; and R^9 is one of hydrogen, methyl, ethyl, butyl, phenyl, benzyl, phenethyl or pyridylmethyl; and

Z^1 and Z^2 are both hydroxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol or diethanolamine.

A fourth preferred subgenus of compounds includes compounds of formula (1a) wherein one of the amino acid side-chains, preferably the side-chain defined by R^2, is an

unnatural amino acid selected from naphthylmethyl, pyridylmethyl and quinolinylmethyl, with quinolinylmethyl being most preferred. Thus, this subgenus includes compounds of formula (1a), wherein at least one R^1, R^2 or R^3 is naphthylmethyl, pyridylmethyl or quinolinylmethyl; provided that the compound is other than isovaleryl-phenylalanine-norvaline-[(naphthylmethyl), (4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-yl)]methylamide or (3-t-butylsulfonyl)propionyl-norvaline-(1-naphthyl, dihydroxyboryl)methylamide.

A fifth preferred subgenus includes compounds of formula (1a) where R, together with R^1, or with R^2 when A is zero, forms a nitrogen containing heterocycle. This subgenus includes compounds having formula (1a), wherein:

R forms together with the adjacent R^1, or when A is zero, forms together with the adjacent R^2, a nitrogen-containing mono-, bi- or tri-cyclic, saturated or partially saturated ring system having 4–14 ring members, and one or two optional substituents selected from the group consisting of keto, hydroxy, aryl, alkoxy and aryloxy;

when A is 2, the R^1 that is not adjacent to N-R is one of hydrogen, alkyl, cycloalkyl, aryl, heterocycle or $—CH_2—R^5$; and when A is 1 or 2, R^2 is one of hydrogen, alkyl, cycloalkyl, aryl, heterocycle or $—CH_2—R^5$, where R^5 is defined as above.

A preferred class of compounds of this embodiment of the invention are those wherein: A is zero; P is hydrogen; X^2 is —C(O)NH—; and R forms together with the adjacent R^2, one of the nitrogen-containing ring systems shown in the above structures; R^3 is C_{1-6} alkyl; and Z^1 and Z^2 are both hydroxy, C_{1-6} alkoxy, or C_{6-10} aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol or diethanolamine. The hydrochloride salts of these compounds are also especially preferred.

Even more preferred are those compounds wherein R forms together with the adjacent R^2, a nitrogen-containing ring system having one of the structures shown above; R^3 is isobutyl; and Z^1 and Z^2 are both hydroxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol or diethanolamine.

Examples of suitable proteasome inhibitors include without limitation the following compounds, as well as pharmaceutically acceptable salts and boronate esters thereof:

N-(4-morpholine)carbonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid,

N-(8-quinoline)sulfonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid,

N-(2-pyrazine)carbonyl-L-phenylalanine-L-leucine boronic acid,

L-proline-L-leucine boronic acid,

N-(2-quinoline)carbonyl-L-homophenylalanine-L-leucine boronic acid,

N-(3-pyridine)carbonyl-L-phenylalanine-L-leucine boronic acid,

N-(3-phenylpropionyl)-L-phenylalanine-L-leucine boronic acid,

N-(4-morpholine)carbonyl-L-phenylalanine-L-leucine boronic acid,

N-(4-morpholine)carbonyl-(O-benzyl)-L-tyrosine-L-leucine boronic acid,

N-(4-morpholine)carbonyl-L-tyrosine-L-leucine boronic acid, and

N-(4-morpholine)carbonyl-[O-(2-pyridylmethyl)]-L-tyrosine-L-leucine boronic acid.

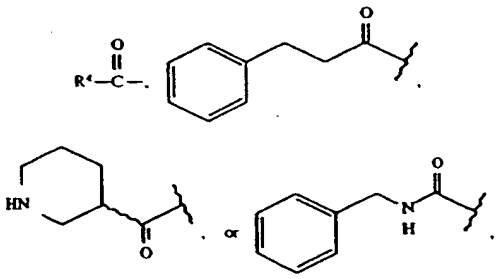
Preferred compounds having formula (2a) include compounds where Y is one of $R^8—C(O)—$, $R^8—SO_2—$, $R^8—NH—C(O)—$ or $R^8—O—C(O)—$, and

R^8 is one of C_{6-10} aryl, C_{6-10} ar(C_{1-6})alkyl, or a 5- 10 membered heteroaryl, any of which can be optionally substituted, or when P is $R^8—C(O)—$, R^8 can also be N-morpholinyl; provided that when Y is $R^8—C(O)—$, then R^8 is other than phenyl, benzyl or C_{1-3} alkyl.

Where R^8 is alkyl, it is preferably alkyl of from 1 to 4 carbon atoms, e.g., methyl, ethyl, propyl, butyl, or isomers thereof. Additionally, where R^8 is alkaryl or aralkyl, the alkyl moiety thereof is also preferably one having from 1 to 4 carbon atoms.

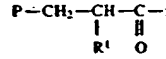
Where R^8 is aryl, it is preferably aryl of from 6 to 10 carbon atoms, e.g., phenyl or naphthyl, which may, if desired, be ring substituted. Additionally, where R^8 is alkaryl, aralkyl, aryloxy, alkaryloxy, or aralkoxy, the aryl moiety thereof is also preferably one having from 5 to 10 carbon atoms, most preferably 6 to 10 carbon atoms. Preferably, the R^8 moiety is a saturated, partially unsaturated or aromatic heterocycle, more preferably an isomeric pyridine ring or morpholine ring.

Y is most preferably one of:



where R^4 is C_{6-12} alkyl.

In an additional preferred embodiment of the present invention, the Y moiety of the proteasome inhibitor of formula (2a) is an isosteric amino acid replacement of formula (3a):

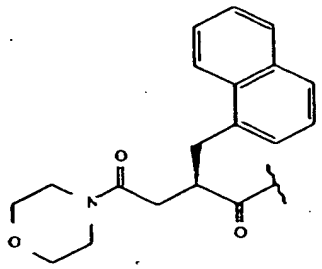


(3a)

where R^1 is as defined for formula (1a) above. Useful and preferred values of R^1 are the same as those defined for formula (1a) above; and

P is one of $R^7—C(O)—$, $R^7—SO_2—$, $R^7—NH—C(O)—$ or $R^7—O—C(O)—$, and R^7 is one of alkyl, aryl, alkaryl, aralkyl, any of which can be optionally substituted, or when Y is $R^7—C(O)—$ or $R^7—SO_2—$, R^7 can also be an optionally substituted 5–10 membered saturated, partially unsaturated or aromatic heterocycle.

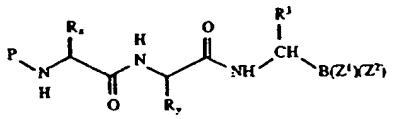
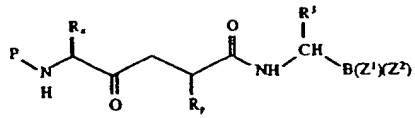
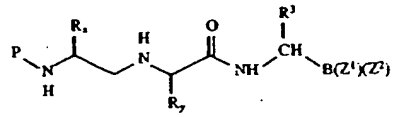
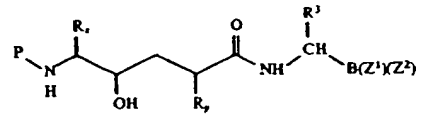
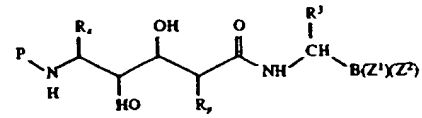
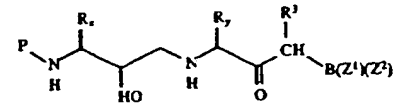
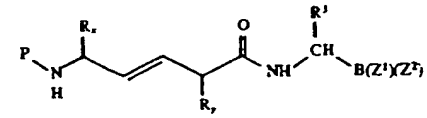
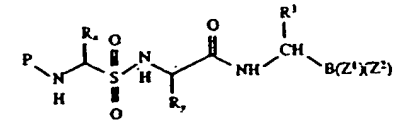
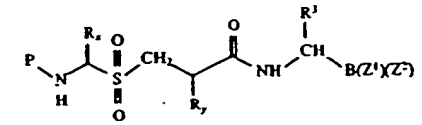
Useful and preferred values of R^7, when R^7 is one of alkyl, aryl, alkaryl, aralkyl, any of which are optionally substituted are as defined for formula (1a) above. When R^7 is optionally substituted 5–10 membered saturated, partially unsaturated or aromatic heterocycle, preferred and useful values are as defined for heteroaryl, unsaturated and partially saturated heterocycle of the R^7 of formula (1a). In this aspect of the invention Y is most preferably:



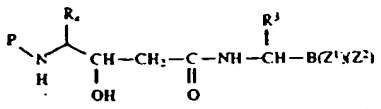
In either embodiment of the compounds of formula (2a), useful and preferred values of R^3 are the same as for formula (1 a) above.

In formula (1a) and (1b), X^1 represents a peptide bond or an isostere that can be used as a peptide bond replacement in the proteasome inhibitors to increase bioavailability and reduce hydrolytic metabolism. As noted above, X^1 can be one of —C(O)NH—, —CH_2—NH—, —CH(OH)—CH(OH)—, —CH(OH)—CH_2—CH(OH)—CH_2—NH—, —CH=CH—, —C(O)—CH_2—, —SO_2—NH—, —SO_2—CH_2— or —CH(OH)—CH_2—C(O)—NH—. Preferably, X^1 is —C(O)—NH—.

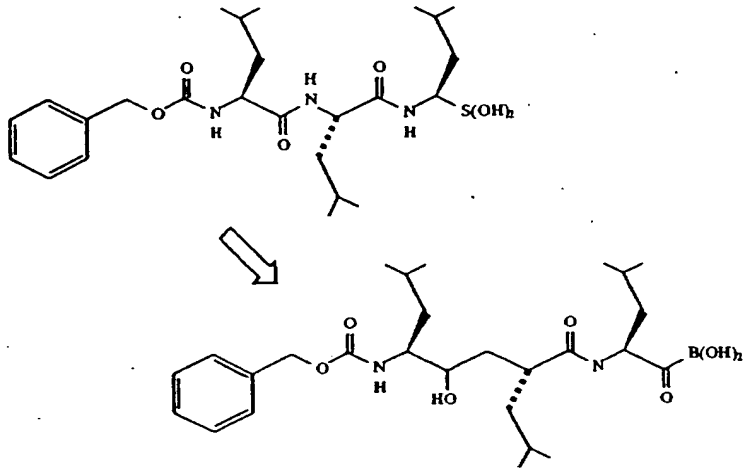
Introduction of these X^1 moieties into the proteasome inhibitors results in the following wherein R_x and R_y have the same definitions as R^1 and R^2, above and p. Z^1, Z^2 and R^3 are defined as above for formula (1a).

	peptide bond
	keto methylene isostere
	reduced peptide bond
	hydroxyethylene isostere
	dihydroxyethylene isostere
	hydroxyethylamine isostere
	alkene isostere
	sulfonamide isostere
	sulfone methylene isostere

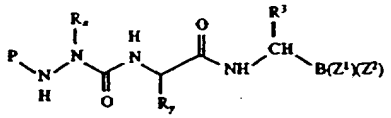
-continued



Thus, for example, if Z-Leu-Leu-Leu-$B(OH)_2$ is found to undergo rapid hydrolytic metabolism to produce Z-Leu-OH and H_2N-Leu-Leu-$B(OH)_2$, the hydroxyethylene isostere can be prepared to eliminate this reaction:

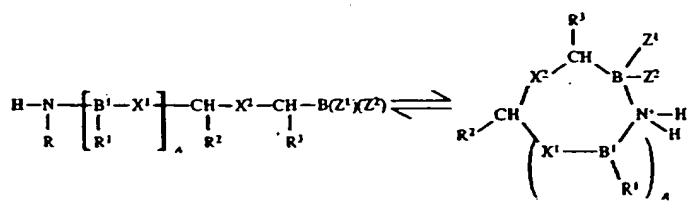


Another group of compounds of the present invention are aza-peptide isosteres. This is the result of the replacement of the a-carbon atom of an amino acid with a nitrogen atom, e.g.,



wherein R_x represents R^1, R_y represents R^2, P, Z^1, Z^2 and R^3 are defined as above for formula (1a) and (1b).

When P and R are both H, formula (1) will exist in equilibrium with a cyclic formula (4), which is considered to be covered by the current invention:



(1) (4)

The above-described boronic ester and acid compounds include both D and L peptidyl configurations. However, L configurations are preferred.

The present invention relates to a method for reducing the rate of muscle protein degradation in a cell comprising contacting the cell with a proteasome inhibitor described above. More specifically, the present invention relates to a method for reducing the rate of loss of muscle mass in an animal comprising contacting cells of the muscle with a proteasome inhibitor described above.

The present invention also relates to a method for reducing the activity of NF-κB in a cell comprising contacting the cell with a proteasome inhibitor described above. More specifically, the present invention also relates to a method for reducing the activity of NF-κB in an animal comprising contacting cells of the animal with a proteasome inhibitor described above.

The present invention also relates to a method for reducing the rate of proteasome-dependent intracellular protein

breakdown comprising contacting cells with a proteasome inhibitor described above. More specifically, the present invention also relates to a method for reducing the rate of intracellular protein breakdown in an animal comprising contacting cells of the animal with the proteasome inhibitor described above.

The present invention further relates to a method of reducing the rate of degradation of p53 protein in a cell comprising administering to the cell a proteasome inhibitor described above. More specifically, the present invention further provides a method of reducing the rate of degradation of p53 protein in an animal (preferably, an animal subjected to DNA damaging drugs or radiation) comprising administering to said animal a proteasome inhibitor described above.

The present invention further relates to a method for inhibiting cyclin degradation in a cell comprising contacting said cells with a proteasome inhibitor described above. More specifically, the present invention relates to a method for inhibiting cyclin degradation in an animal comprising contacting cells of said animal with a proteasome inhibitor described above.

The present invention also provides a method for treating cancer, psoriasis, restenosis, or other cell proliferative diseases in a patient comprising administering to the patient a proteasome inhibitor described above.

The present invention also relates to a method for inhibiting antigen presentation in a cell comprising administering to the cell a proteasome inhibitor described above. More specifically, the present invention relates to a method for inhibiting antigen presentation in animal comprising administering to the animal a proteasome inhibitor described above.

The present invention further provides a method for inhibiting inducible NF-κB dependent cell adhesion in an animal comprising administering to said animal a proteasome inhibitor described above.

The present invention also provides a method for inhibiting HIV infection in an animal comprising administering to said animal a proteasome inhibitor described above.

The "animals" referred to herein are preferably mammals. Both terms are intended to include humans.

Preferably, the methods described above deliver the proteasome inhibitor by either contacting cells of the animal with a proteasome inhibitor described above or by administering to the animal a proteasome inhibitor described above.

The compounds of the present invention inhibit the functioning of the proteasome. This proteasome-inhibition activity results in the inhibition or blocking of a variety of intracellular functions. In particular, inhibition of proteasome function inhibits the activation or processing of transcription factor NF-κB. NF-κB plays a central role in the regulation of a diverse set of genes involved in the immune and inflammatory responses. Inhibition of proteasome function also inhibit the ubiquitination/proteolysis pathway. This pathway catalyzes selective degradation of highly abnormal proteins and short-lived regulatory proteins. The ubiquitination proteolysis pathway also is involved in the processing of internalized cellular or viral antigens into antigenic peptides that bind to MHC-I molecules. Thus, the proteasome inhibitors of the present invention can be used in reducing the activity of the cytosolic ATP-ubiquitin-dependent proteolytic system in a number of cell types.

The inhibitors can be used in vitro or in vivo. They can be administered by any number of known routes, including orally, intravenously, intramuscularly, subcutaneously, intrathecally, topically, and by infusion (Platt et al., U.S. Pat. No. 4,510,130; Badalamente et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:5983–5987 (1989); Staubli et al., *Brain Research* 444:153–158 (1988)) and will generally be administered in combination with a physiologically acceptable carrier (e.g., physiological saline). The effective quantity of inhibitor given will be determined empirically and will be based on such considerations as the particular inhibitor used, the condition of the individual, and the size and weight of the individual. It is to be expected that the general end-use application dose range will be about 0.01 to 100 mg per kg per day, preferably 0.1 to 75 mg per kg per day for an effective therapeutic effect.

The present invention relates to a method of inhibiting (reducing or preventing) the accelerated or enhanced proteolysis that occurs in atrophying muscles and is known to be due to activation of a nonlysosomal ATP-requiring process in which ubiquitin plays a critical role.

Inhibition of the ATP-ubiquitin-dependent pathway is a new approach for treating the negative nitrogen balance in catabolic states. This can be effected through use of an inhibitor of the present invention, resulting in reduction of loss of muscle mass in conditions in which it occurs. Excessive protein loss is common in many types of patients, including individuals with sepsis, burns, trauma, many cancers, chronic or systemic infections, neuromotor degenerative disease, such as muscular dystrophy, acidosis, or spinal or nerve injuries. It also occurs in individuals receiving corticosteroids, and those in whom food intake is reduced and/or absorption is compromised. Moreover, inhibitors of the protein breakdown pathway could possibly be valuable in animals, e.g., for combating "shipping fever", which often leads to a major weight loss in cattle or pigs.

The accelerated proteolysis evident in atrophy of skeletal muscles upon denervation or fasting is catalyzed by the nonlysosomal ATP-dependent degradative pathway. It has been shown that in a variety of catabolic states (e.g., denervation, fasting, fever, certain endocrinopathies or metabolic acidosis) muscle wasting is due primarily to accelerated protein breakdown and, in addition, that the increased proteolysis results from activation of the cytosolic ATP-ubiquitin-dependent proteolytic system, which previously had been believed to serve only in the rapid elimination of abnormal proteins and certain short-lived enzymes. The discovery that this pathway is responsible for the accelerated proteolysis in these catabolic states is based on studies in which different proteolytic pathways were blocked or measured selectively in incubated muscles, and the finding of increased mRNA for components of this pathway (e.g., for ubiquitin and proteasome subunits) and increased levels of ubiquitin-protein conjugates in the atrophying muscles. The nonlysosomal ATP-ubiquitin-dependent proteolytic process increases in muscle in these conditions and is responsible for most of the accelerated proteolysis that occurs in atrophying muscles. There is a specific increase in ubiquitin mRNA, induction of mRNA for proteasome and increased ubiquitinated protein content in atrophying muscles that is not seen in non-muscle tissue under the same conditions.

The inhibitors of the present invention can be used to reduce (totally or partially) the nonlysosomal ATP-dependent protein degradation shown to be responsible for most of the increased protein degradation that occurs during fasting, denervation, or disuse (inactivity), steroid therapy, febrile infection, and other conditions.

One approach to testing drug candidates for their ability to inhibit the ATP-ubiquitin-dependent degradative process is to measure proteolysis in cultured cells (Rock et al., *Cell* 78:761 (1994)). For example, the degradation of long-lived intracellular proteins can be measured in mouse C2C12 myoblast cells. Cells are incubated with ^{35}S-methionine for 48 hours to label long-lived proteins and then chased for 2 hours with medium containing unlabeled methionine. After the chase period, the cells are incubated for 4 hours in the presence or absence of the test compound. The amount of

protein degradation in the cell can be measured by quantitating the trichloroacetic acid soluble radioactivity released from the pre-labeled proteins into the growth medium (an indicator of intracellular proteolysis).

Inhibitors can also be tested for their ability to reduce muscle wasting in vivo. Urinary excretion of the modified amino acid 3-methyl histidine (3-MH) is probably the most well characterized method for studying myofibrillar protein degradation in vivo (see Young and Munro. *Federation Proc.* 37:229–2300 (1978)). 3-Methylhistidine is a post-translationally modified amino acid which cannot be reutilized for protein synthesis, and it is only known to occur in actin and myosin. It occurs in actin isolated from all sources, including cytoplasmic actin from many different cell types. It also occurs in the myosin heavy chain of fast-twitch (white, type II) muscle fibers, but it is absent from myosin of cardiac muscle and myosin of slow-twitch (red, type I) muscle fibers. Due to its presence in actin of other tissues than skeletal muscle, other tissues will contribute to urinary 3-MH. Skeletal muscle has been estimated to contribute 38–74% of the urinary 3-MH in normal rats and 79–86% of the urinary 3-MH in rats treated with corticosterone (100 mg/kg/day subcutaneously) for 2–4 days (Millward and Bates. *Biochem. J.* 214.607–615 (1983); Kayali, et al., *Am. J. Physiol.* 252:E621-E626 (1987)).

High-dose glucocorticoid treatment is used to induce a state of muscle wasting in rats. Treating rats with daily subcutaneous injections of corticosterone (100 mg/kg) causes an increase of approximately 2-fold in urinary 3-MH. The increase in excretion of 3-MH is transient, with a peak increase after 2–4 days of treatment and a return to basal values after 6–7 days of treatment (Odedra, et al., *Biochem. J* 214:617–627 (1983); Kayali, et al., *Am. J Physiol.* 252E621-E626 (1987)). Glucocorticoids have been shown to activate the ATP-ubiquitin-dependent proteolytic pathway in skeletal muscle (Wing and Goldberg. *Am. J Physiol.* 264:E668-E676 (1993)) and proteasome inhibitors are therefore expected to inhibit the muscle wasting that occurs after glucocorticoid treatment.

The proteasome inhibitors can be administered alone or in combination with another inhibitor or an inhibitor of another pathway (e.g., a lysosomal or Ca^{++}-dependent pathway) responsible for loss of muscle mass.

Use of proteasome inhibitors as agents that selectively protect normal cells from DNA damage during radiation and chemotherapy treatment of tumors

The inhibitors of the present invention will block the degradation of the tumor suppressor protein p53. This protein is degraded by the ATP ubiquitin dependent proteolysis by the proteasome (see Scheffner et al., *Cell* 75:495–505 (1993)).

Studies of p53 knockout mice indicate an important role for p53 in reducing incidence of tumors (Donehower et al., *Nature* 356:215–221 (1992)). In normal cells expressing wild type, unmutated p53, the basal levels of p53 are very low due to very rapid degradation of p53 protein. However, expression of p53 protein in normal cells is stimulated in response to radiation and drugs that induce DNA damage (Kastan et al., *Cancer Res.* 51.6304–6311 (1991)). These induced high levels of wild type, unmutated p53 induce arrest of normal cell proliferation at the G1 stage of the cell cycle (Kastan et al., supra; Kuerbitz, *PNAS* 89:7491–7495 (1992)). This arrest of cell proliferation permits repair of damaged DNA. By contrast, in tumor cells expressing mutant forms of p53, DNA damaging drugs or radiation do not induce cell cycle arrest (Kastan et al., supra; Kastan et al., *Cell* 71:587–597 (1992)). Consequently, tumor cells are selectively damaged by radiation and cytotoxic drugs.

The selective arrest response of normal cells by inducing p53 suggests that enhancing the p53 response can allow the treatment of the tumor with higher/more prolonged tumoricidal doses of radiation or antineoplastic drugs. The idea that induction of p53 by a non toxic agent as an adjunct to radiotherapy has been reported previously (Lane, *Nature* 358:15–16 (1992)), but a method for reducing it to practice was not described.

The use of proteasome inhibitors provides a method for augmenting the expression of p53 in normal cells by preventing its degradation by the proteasome. An example of this would be the systemic administration of proteasome inhibitor at a sufficient dose to inhibit p53 degradation by the proteasome during the treatment of the tumor with cytotoxic drugs or radiation. This will prolong and increase the levels of p53 expression in normal cells and will enhance the arrest of normal cell proliferation, reducing their sensitivity to higher doses of radiation or cytotoxic drugs. Administration of proteasome inhibitors would therefore permit exposing the tumor to higher doses of radiation, enhancing the killing of tumor cells. Thus, proteasome inhibitors can be used as adjuvants to therapy with tumoricidal agents, such as radiation and cytotoxic drugs.

Topical application of proteasome inhibitors to enhance p53 expression in skin

The expression of p53 in normal skin is induced by exposure of the skin to UV irradiation, which inhibits DNA replication that is needed for cell division (Maltzman et al., *Mol. Cell. Biol.* 4.1689 (1984); Hall et al., *Oncogene* 8.203–207 (1993)). This protects normal skin from chromosomal DNA damage by allowing time for DNA repair before DNA replication.

Defects in the p53 response pathway, such as seen with Ataxia Telangiectasia, result in increased susceptibility to ionizing radiation-induced skin tumors (Kastan et al., *Cell* 71:587–597 (1992)). It is well established that exposure of normal individuals increases the risk for many kinds of skin cancers. This risk can be diminished by UV filtering chemicals in skin creams. Another approach would be to promote the resistance of the DNA in skin cells to UV damage by the topical application of agents that enhance the skin's expression of p53 in response to UV light. Inhibiting p53 degradation by the topical application of proteasome inhibitors provides a method to enhance the p53 response.

One preferred embodiment of the present invention is the topical application of proteasome inhibitors to reduce the acknowledged risk of skin cancers that results from the treatment of psoriasis using UV light, which is often combined with psoralens or coal tar. Each of these agents can induce DNA damage.

Use of proteasome inhibitors to reduce the activity of NF-κB

NF-κB exists in an inactive form in the cytoplasm complexed with an inhibitor protein, IκB. In order for the NF-κB to become active and perform its function, it must enter the cell nucleus. It cannot do this, however, until the IκB portion of the complex is removed, a process referred to by those skilled in the art as the activation of, or processing of, NF-κB. In some diseases, the normal performance of its function by the NF-κB can be detrimental to the health of the patient. For example, as mentioned above, NF-κB is essential for the expression of the human immunodeficiency virus (HIV). Accordingly, a process that would prevent the activation of the NF-κB in patients suffering from such diseases could be therapeutically beneficial. The inhibitors employed in the practice of the present invention are capable of preventing this activation. Thus, blocking NF-κB activity could have important application in various areas of medicine, e.g., inflammation, through the inhibition of expression of inflammatory cytokines and cell adhesion molecules, (ref. Grilli et al., *International Review of Cytology* 143: 1–62 (1993)) sepsis, AIDS, and the like.

More specifically, the activity of NF-κB is highly regulated (Grilli et al., *International Review of Cytology* 143: 1–62 (1993); Beg et al., *Genes and Development*

7:2064–2070 (1993)). NF-κB comprises two subunits. p50 and an additional member of the rel gene family. e.g., p65 (also known as Rel A). In most cells. the p50 and p65 are present in an inactive precursor form in the cytoplasm. bound to IκB. In addition. the p50 subunit of NF-κB is generated by the proteolytic processing of a 105 kD precursor protein NF-κB$_1$ (p105). and this processing is also regulated. The sequence of the N-terminal 50 kD portion of p105 is similar to that of p65 and other members of the rel gene family (the rel homology domain). By contrast. the C-terminal 55 kD of p105 bears a striking resemblance to IκB-α (also known as MAD3). Significantly. unprocessed p105 can associate with p65 and other members of the rel family to form a p65/p105 heterodimer. Processing of p105 results in the production of p50. which can form the transcriptionally active p50/p65 heterodimer. The C-terminal IκB-α-homologous sequence of p105 is rapidly degraded upon processing.

There is another rel-related protein. NF-κB$_2$ (p100). that is similar to p105 in that it. too. is processed to a DNA binding subunit. p52 (Neri et al., *Cell* 67:1075 (1991); Schmid et al., *Nature* 352:733 (1991); Bours et al., *Molecular and Cellular Biology* 12:685 (1992); Mercurio et al., *DNA Cell Biology* 11:523 (1992)). Many of the structural and regulatory features of p100 are similar to p105. In addition. the p100 protein can also form a heterodimer with p65 and other rel family members.

In summary. the transcriptional activity of heterodimers consisting of p50 and one of the many rel family proteins. such as p65. can be regulated by at least two mechanisms. First. the heterodimers associate with IκB-α to form an inactive ternary cytoplasmic complex. Second. the rel family members associate with p105 and p100 to form inactive complexes. The ternary complex can be activated by the dissociation and destruction of IκB-α. while the p65/p105 and p65/p100 heterodimer can be activated by processing p105 and p100. respectively.

The dissociation of IκB-α can be induced by a remarkably large number of extracellular signals. such as lipopolysaccharides. phorbol esters. TNF-α. and a variety of cytokines. The IκB-α is then rapidly degraded. Recent studies suggest that p105 and p100 processing can also be induced by at least some of these extracellular signals.

Studies have demonstrated that p 105 or a truncated form of p 105 (p60Tth) can be processed to p50 in vitro (Fan et al., *Nature* 354:395–398 (1991)). Certain of the requirements and characteristics of this in vitro processing reaction (e.g., ATP/Mg^{++} dependency) implicated the involvement of the ubiquitin-mediated protein degradation pathway (Goldberg. *Eur. J. Biochem.* 203:9–23 (1992). Hershko et al., *Annu. Rev. Biochem.* 61:761–807 (1992)).

The proteasome is required for the processing of p105 to p50. p105/p60Tth proteins are not processed in mammalian cell cytoplasmic extracts depleted of proteasome activity. However. addition of purified 26S proteasomes to these depleted extracts restores the processing activity. Additionally. specific inhibitors of the proteasome block the formation of p50 in mammalian cell extracts and in vivo. Also. mammalian p105 is processed to p50 in *Saccharomyces cerevisiae* in vivo. and a mutant deficient in the chymotrypsin-like activity of the proteasome showed a significant decrease in p 105 processing. p60Tth is ubiquitinated in vitro and this ubiquitination is a pre-requisite for p105 processing.

As mentioned above. the C-terminal half of the p105 (p105C) is rapidly degraded during the formation of p50 and the sequence of p105C is remarkably similar to that of IκB. IκB-α is rapidly degraded in response to NF-κB inducers and this degradation has been shown to be necessary for the activation (Mellits et al., *Nucleic Acids Research* 21(22):5059–5066 (1993); Henkel et al., *Nature* 365:182–185 (1993); Beg et al., *Molecular and Cellular Biology* 13(6):3301–3310 (1993)). IκB-α degradation and the activation of NF-κB are also blocked by inhibitors of proteasome function or ubiquitin conjugation (Palombella et al., *Cell* 78:773–785 (1994)).

Accordingly. the proteasome plays an essential role in the regulation of NF-κB activity. First. the proteasome is required for the processing of p105 and possibly p100. The degradation of the inhibitory C-terminus can also require the proteasome. Second. the proteasome appears to be required for the degradation of IκB-α in response to extracellular inducers.

The present invention relates to a method for reducing the activity of NF-κB in an animal comprising contacting cells of the animal with inhibitors of proteasome function.

Compounds can be tested for their ability to inhibit the activation of NF-κB by means of a DNA binding assay (Palombella, et al., *Cell* 78.773 (1994)). Whole-cell extracts are prepared from untreated or TNF-α treated cells that have been pretreated for 1 hour with the test compound. The DNA binding activity of NF-κB is measured by an electrophoretic mobility shift assay using the PRDII probe from the human IFN-β gene promoter.

As an indirect measure of NF-κB activation. the cell-surface expression of E-selectin. I-CAM-1. and V-CAM-1 on primary human umbilical vein endothelial cells (HUVECs) can be determined by means of a cell surface fluorescent immuno-binding assay. Because E-selectin. I-CAM-1. and V-CAM-1 are under the regulatory control of NF-κB. inhibition of NF-κB activation results in reduced levels of these adhesion molecules on the cell surface.

Compounds can also be tested for their ability to inhibit a delayed-type hypersensitivity response in mice. Contact hypersensitivity is a manifestation of an in vivo T-cell mediated immune response (Friedmann. *Curr. Opinion Immunology*, 1:690–693 (1989)). Although the exact molecular mechanisms that regulate the cellular interactions and vascular changes involved in the response remain obscure. it is clear that the process is dependent upon the interplay of soluble mediators. adhesion molecules. and the cytokine network (Piguet. et al., *J Exp. Med.* 173:673–679 (1991); Nickoloff. et al. *J. Invest. Dermatol.* 94:151S-157S (1990)). NF-κB. by mediating events such as the production of cytokines and the induction and utilization of cell-surface adhesion molecules. is a central and coordinating regulator involved in immune responses.

The compounds of formula (1b) or (2b) can be used to treat chronic or acute inflammation that is the result of transplantation rejection. arthritis. rheumatoid arthritis. infection. dermatosis. inflammatory bowel disease. asthma. osteoporosis. osteoarthritis and autoimmune disease. Additionally. inflammation associated with psoriasis and restenosis can also be treated.

The term "treatment of inflammation" or "treating inflammation" is intended to include the administration of compounds of the present invention to a subject for purposes which can include prophylaxis. amelioration. prevention or cure of an inflammatory response. Such treatment need not necessarily completely ameliorate the inflammatory response. Further. such treatment can be used in conjunction with other traditional treatments for reducing the inflammatory condition known to those of skill in the art.

The proteasome inhibitors of the invention can be provided as a "preventive" treatment before detection of an inflammatory state. so as to prevent the same from developing in patients at high risk for the same. such as. for example. transplant patients.

In another embodiment. efficacious levels of the proteasome inhibitors of the invention are administered so as to provide therapeutic benefits against the secondary harmful inflammatory effects of inflammation. By an "efficacious

level" of a composition of the invention is meant a level at which some relief is afforded to the patient who is the recipient of the treatment. By an "abnormal" host inflammatory condition is meant an level of inflammation in the subject at a site which exceeds the norm for the healthy medical state of the subject, or exceeds a desired level. By "secondary" tissue damage or toxic effects is meant the tissue damage or toxic effects which occur to otherwise healthy tissues, organs, and the cells therein, due to the presence of an inflammatory response, including as a result of a "primary" inflammatory response elsewhere in the body.

Amounts and regimens for the administration of proteasome inhibitors and compositions of the invention can be determined readily by those with ordinary skill in the clinical art of treating inflammation-related disorders such as arthritis, tissue injury and tissue rejection. Generally, the dosage of the composition of the invention will vary depending upon considerations such as: type of pharmaceutical composition employed; age; health; medical conditions being treated; kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired; extent of tissue damage; gender; duration of the symptoms; and, counter indications, if any, and other variables to be adjusted by the individual physician. A desired dosage can be administered in one or more applications to obtain the desired results. Pharmaceutical compositions containing the proteasome inhibitors of the invention can be provided in unit dosage forms.

Thus, the proteasome inhibitors are useful for treating such conditions as tissue rejection, arthritis, local infections, dermatoses, inflammatory bowel diseases, autoimmune diseases, etc. The proteasome inhibitors of the present invention can be employed to prevent the rejection or inflammation of transplanted tissue or organs of any type, for example, heart, lung, kidney, liver, skin grafts, and tissue grafts.

Compounds of the present invention inhibit the growth of cancer cells. Thus, the compounds can be employed to treat cancer, psoriasis, restenosis or other cell proliferative diseases in a patient in need thereof.

By the term "treatment of cancer" or "treating cancer" is intended description of an activity of compounds of the present invention wherein said activity prevents or alleviates or ameliorates any of the specific phenomena known in the art to be associated with the pathology commonly known as "cancer." The term "cancer" refers to the spectrum of pathological symptoms associated with the initiation or progression, as well as metastasis, of malignant tumors. By the term "tumor" is intended, for the purpose of the present invention, a new growth of tissue in which the multiplication of cells is uncontrolled and progressive. The tumor that is particularly relevant to the invention is the malignant tumor, one in which the primary tumor has the properties of invasion or metastasis or which shows a greater degree of anaplasia than do benign tumors.

Thus, "treatment of cancer" or "treating cancer" refers to an activity that prevents, alleviates or ameliorates any of the primary phenomena (initiation, progression, metastasis) or secondary symptoms associated with the disease. Cancers that are treatable are broadly divided into the categories of carcinoma, lymphoma and sarcoma. Examples of carcinomas that can be treated by the composition of the present invention include, but are not limited to: adenocarcinoma, acinic cell adenocarcinoma, adrenal cortical carcinomas, alveoli cell carcinoma, anaplastic carcinoma, basaloid carcinoma, basal cell carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, renaladinol carcinoma, embryonal carcinoma, anometroid carcinoma, fibrolamolar liver cell carcinoma, follicular carcinomas, giant cell carcinomas, hepatocellular carcinoma, intraepidermal carcinoma, intraepithelial carcinoma, leptomanigio carcinoma, medullary carcinoma, melanotic carcinoma, menigual carcinoma, mesometonephric carcinoma, oat cell carcinoma, squamal cell carcinoma, sweat gland carcinoma, transitional cell carcinoma, and tubular cell carcinoma. Sarcomas that can be treated by the composition of the present invention include, but are not limited to: amelioblastic sarcoma, angiolithic sarcoma, botryoid sarcoma, endometrial stroma sarcoma, ewing sarcoma, fascicular sarcoma, giant cell sarcoma, granulositic sarcoma, immunoblastic sarcoma, juxaccordial osteogenic sarcoma, coppices sarcoma, leukocytic sarcoma (leukemia), lymphatic sarcoma (lympho sarcoma), medullary sarcoma, myeloid sarcoma (granulocitic sarcoma), austiogenci sarcoma, periosteal sarcoma, reticulum cell sarcoma (histiocytic lymphoma), round cell sarcoma, spindle cell sarcoma, synovial sarcoma, and telangiectatic audiogenic sarcoma. Lymphomas that can be treated by the composition of the present invention include, but are not limited to: Hodgkin's disease and lymphocytic lymphomas, such as Burkitt's lymphoma, NPDL, NML, NH and diffuse lymphomas.

The compounds of formulae (1b) and (2b) appear to be particularly useful in treating metastases.

Amounts and regimens for the administration of proteasome inhibitors and compositions of the invention can be determined readily by those with ordinary skill in the clinical art of treating cancer-related disorders such as the primary phenomena (initiation, progression, metastasis) or secondary symptoms associated with the disease. Generally, the dosage of the composition of the invention will vary depending upon considerations such as: type of composition employed; age; health; medical conditions being treated; kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired; extent of tissue damage; gender; duration of the symptoms; and, counter indications, if any, and other variables to be adjusted by the individual physician. A desired dosage can be administered in one or more applications to obtain the desired results. Pharmaceutical compositions containing the proteasome inhibitors of the invention can be provided in unit dosage forms.

The present invention will now be illustrated by the following examples, which are not intended to be limiting in any way.

EXAMPLES

Most compounds of formulas (1a), (1b), (2a) or (2b) were prepared according to the general reaction sequence depicted in Scheme 1. R^2 and R^3 are as defined above for formulas (1b) and (2b). PG represents an amino-group-protecting moiety. The general procedures employed for each compound are summarized in Table 1, and detailed descriptions of these procedures are provided in the Examples. Syntheses that do not conform to the general reaction sequence are described in full in the Examples. (1S,2S,3R,5S)-Pinanediol leucine boronate trifluoroacetate salt was prepared as previously reported (Kettner, C. A.; Shenvi, A. B. *J Biol. Chem.* 259–15106 (1984)). N-Protected (Boc-, Cbz-, or Fmoc-) amino acids were commercially available or were prepared from the corresponding free amino acid by standard protection methods, unless otherwise described in the Examples. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent), or O-(1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU) were employed as coupling reagents (Sheehan, J. C. et al., *J Am. Chem. Soc.* 87:2492 (1965); Castro, B., et al., *Synthesis* 11:751 (1976); *Tetrahedron Lett.* 30:1927 (1989)). All compounds were characterized by proton nuclear magnetic resonance (NMR) spectroscopy. The purity of the products was verified by thin layer chromatography and by high performance liquid chromatography (HPLC).

Scheme I

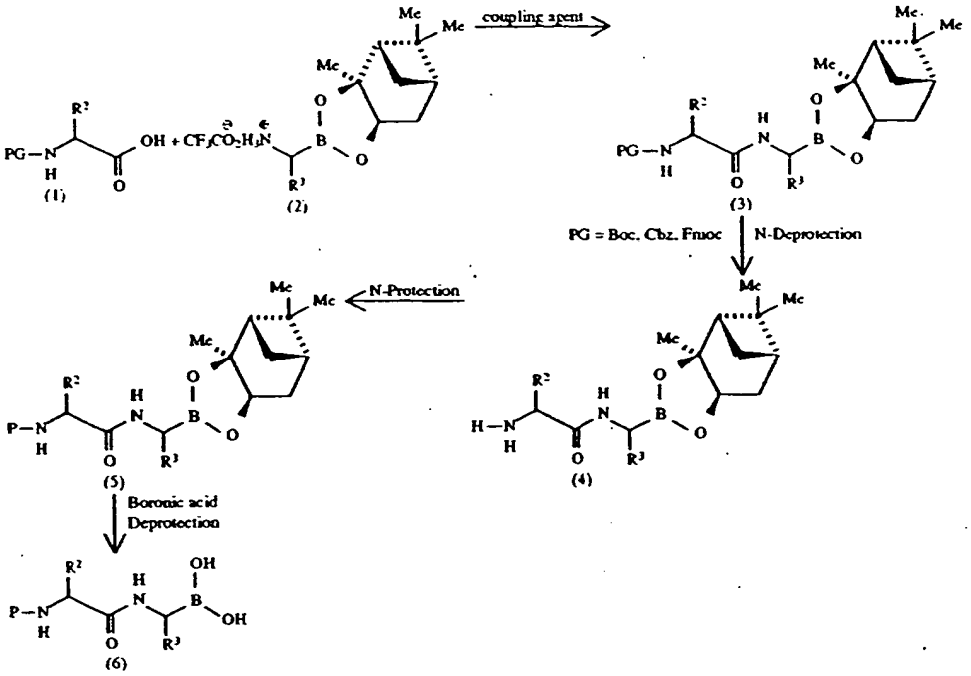


TABLE I

Synthesis of Boronic Ester and Acid Compounds

Compound	Coupling Agent	Boronic Acid Deprotection*	N-Terminal Protection
MG-261	EDC	—	—
MG-262	EDC	A	—
MG-264	BOP	—	—
MG-267	EDC	—	—
MG-268	EDC	A	NaH, MeI
MG-270	EDC	A	—
MG-272	EDC	A	—
MG-273	EDC	A, B	RC(O)Cl
MG-274	BOP	A	—
MG-278	EDC	A	RC(O)Cl
MG-282	EDC	A	—
MG-283	BOP	A	Ac_2O
MG-284	—	B	RC(O)Cl
MG-285	BOP	A	RC(O)Cl
MG-286	EDC	A, B	RC(O)Cl
MG-287	EDC	B	Ac_2O
MG-288	EDC	A	RC(O)Cl
MG-289	EDC	B	$RS(O)_2Cl$
MG-290	EDC	B	Ac_2O
MG-291	EDC	B	$RS(O)_2Cl$
MG-292	BOP	B	RC(O)Cl
MG-293	TBTU	B	RC(O)Cl
MG-294	EDC	B	—
MG-295	BOP	B	$RS(O)_2Cl$
MG-296	EDC	B	$RS(O)_2Cl$
MG-297	EDC	B	$RS(O)_2Cl$
MG-298	EDC	B	RC(O)Cl
MG-299	EDC	B	RC(O)Cl
MG-300	EDC	B	RC(O)Cl
MG-301	BOP	B	Ac_2O
MG-302	EDC	B	—
MG-303	EDC	B	HCl, ether

TABLE I-continued

Synthesis of Boronic Ester and Acid Compounds

Compound	Coupling Agent	Boronic Acid Deprotection*	N-Terminal Protection
MG-304	TBTU	B	—
MG-305	EDC	B	RC(O)Cl
MG-306	TBTU	B	RC(O)Cl
MG-307	TBTU	B	RC(O)Cl
MG-308	TBTU	B	RC(O)Cl
MG-309	TBTU	B	RC(O)Cl
MG-310	BOP	B	Ac_2O
MG-311	BOP	B	HCl, dioxane
MG-312	EDC	B	RC(O)Cl
MG-313	—	B	RCO_2H, TBTU
MG-314	TBTU	B	RC(O)Cl
MG-315	BOP	B	RC(O)Cl
MG-316	BOP	B	
MG-319	TBTU	B	
MG-321	TBTU	B	RC(O)Cl
MG-322	TBTU	B	RC(O)Cl
MG-323	—	B	Ac_2O
MG-325	TBTU	B	RCO_2H, TBTU
MG-328	TBTU	B	RC(O)Cl
MG-329	TBTU	B	RC(O)Cl
MG-332	TBTU	B	NaH, MeI
MG-333	TBTU	B	NaH, MeI
MG-334	TBTU	B	NaH, MeI
MG-336	TBTU	B	RC(O)Cl
MG-337	TBTU	B	HCl, dioxane
MG-338	EDC	B	RC(O)Cl
MG-339	TBTU	B	HCl, dioxane
MG-340	TBTU	B	HCl, dioxane
MG-341	TBTU	B	RCO_2H, TBTU
MG-342	—	B	RNH_2, TBTU
MG-343	TBTU	B	RCO_2H, TBTU
MG-344	BOP	B	Ac_2O

TABLE 1-continued

Synthesis of Boronic Ester and Acid Compounds

Compound	Coupling Agent	Boronic Acid Deprotection*	N-Terminal Protection
MG-345	EDC	B	RC(O)Cl
MG-346	EDC	B	RC(O)Cl
MG-347	EDC	B	$RS(O)_2Cl$
MG-348	TBTU	B	HCl, dioxane
MG-349	TBTU	B	HCl, dioxane
MG-350	TBTU	B	$PhCH_2NCO$
MG-351	EDC	B	—
MG-352	TBTU	B	RCO_2H, TBTU
MG-353	TBTU	B	RC(O)Cl
MG-354	BOP	B	$RS(O)_2Cl$
MG-356	TBTU	B	—
MG-357	TBTU	B	HCl, dioxane
MG-358	TBTU	B	RC(O)Cl
MG-359	TBTU	B	HCl, dioxane
MG-361	TBTU	B	RCO_2H, TBTU
MG-362	—	B	$PhCH_2NCO$
MG-363	TBTU	B	HCl, dioxane
MG-364	—	B	RCO_2H, TBTU
MG-366	TBTU	B	HCl, dioxane
MG-367	—	B	RC(O)Cl
MG-368	EDC	B	TBTU
MG-369	TBTU	B	HCl, dioxane
MG-380	TBTU	B	$RS(O)_2Cl$
MG-382	TBTU	B	RCO_2H, TBTU
MG-383	TBTU	B	RCO_2H, TBTU
MG-385	TBTU	B	HCl, dioxane
MG-386	TBTU	B	HCl, dioxane
MG-387	TBTU	B	RC(O)Cl

*A = $NaIO_4$, NH_4OAc, acetone-water; B = i-BuB$(OH)_2$, 1N HCl, MeOH-hexane. See Examples for detailed descriptions of procedures.

EXAMPLE 1

N-(4-Morpholine)carbonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid [MG-273]

A. (1S,2S,3R,5S)-Pinanediol N-Boc-β-(1-naphthlyl)-L-alanine-L-leucine boronate

To a solution of (1S,2S,3R,5S)-pinanediol leucine boronate trifluoroacetate salt (664 mg, 1.76 mmol) and N-Boc-β-(1-naphthyl)-L-alanine (555 mg, 1.76 mmol) in DMF (10 mL) at 0° C. was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (404 mg, 2.11 mmol), 1-hydroxybenzotriazole monohydrate (HOBT) (285 mg, 2.11 mmol), and N-methylmorpholine (NMM) (0.3 mL, 2.64 mmol). The mixture was allowed to warm to room temperature and stir overnight. The reaction was quenched with water (100 mL), and the mixture was extracted with CH_2Cl_2 (4×25 mL). The combined organic layers were washed with 5% aqueous HCl and saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, filtered, and concentrated to give a yellow oil. Water was added and the resultant gummy precipitate was extracted with ether (3×25 mL). The organic layer was dried (anhydrous $MgSO_4$), filtered, and concentrated to afford the title compound (202 mg) as a white foam.

B. (1S,2S,3R,5S)-Pinanediol β-(1-Naphthyl)-L-alanine-L-leucine boronate trifluoroacetate salt

To a solution of the product of Example 1A (930 mg, 1.38 mmol) in CH_2Cl_2 (10 mL) at 0° C. was added trifluoroacetic acid (5 mL) and thioanisole (1 mL). The reaction mixture was allowed to warm to room temperature. After 4 h, the reaction mixture was concentrated to dryness and dried in vacuo. The residue was used in the next reaction without further purification.

C. (1S,2S,3R,5S)-Pinanediol N-(4-morpholine) carbonyl-β-(1-naphthlyl)-L-alanine-L-leucine boronate

4-Morpholinecarbonyl chloride (50 mL, 0.42 mmol) and triethylamine (150 mL, 1.08 mmol) were added to a solution of the product of Example 1B (0.25 g, 0.36 mmol) in CH_2Cl_2 (6 mL). After 24 h, additional morpholinecarbonyl chloride (50 mL) and triethylamine (150 mL) were added. After 2 days total reaction time, the reaction mixture was diluted with EtOAc, washed with 1N HCl and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. Purification by flash chromatography (elution with 1:2 EtOAc/hexanes and 4:4:1 hexanes/EtOAc/MeOH) afforded the title compound (124 mg).

D. N-(4-Morpholine)carbonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid

To a stirred solution of the product of Example 1C (124 mg, 0.21 mmol) in acetone (10 mL) was added aqueous NH_4OAc (0.1 N, 5 mL, 1.0 mmol), followed by $NaIO_4$ (120 mg, 0.21 mmol). The reaction mixture was stirred at room temperature for 72 h, and then the acetone was evaporated. The aqueous layer was acidified to pH 3 with 1N HCl and extracted with EtOAc (3×20 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (elution with 1:1 hexane/EtOAc, 2:2:1 hexanes/EtOAc/MeOH, and 1:1: few drops MeOH:EtOAc:HOAc) to give the title compound (29 mg).

EXAMPLE 2

N-Cbz-L-Leucine-L-leucine boronic acid [MG-274]

A. (1S,2S,3R,5S)-Pinanediol N-Cbz-L-leucine-L-leucine boronate

Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 827 mg, 1.87 mmol) was added in one portion to a mixture of (1S,2S,3R,5S)-pinanediol leucine boronate trifluoroacetate salt (595 mg, 1.58 mmol), N-Cbz-L-leucine (500 mg, 1.87 mmol) in acetonitrile (30 mL) at room temperature. The mixture was stirred at room temperature for 2 hours. The reaction was quenched with brine (50 mL) and the mixture was extracted with EtOAc (3×50 mL). The combined organic layers were washed with aqueous 5% HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, and then dried (anhydrous $MgSO_4$), filtered, and concentrated. The residue was purified by silica gel chromatography (elution with 20–30% acetone/hexanes) to afford the title compound (539 mg).

B. N-Cbz-L-Leucine-L-leucine boronic acid

By a procedure analogous to that described in Example 1D, the compound of Example 2A above (539 mg) was deprotected by treatment with sodium metaperiodate (1.2 g, 5.61 mmol) and aqueous NH_4OAc (0.1 N, 10 mL, 1.0 mmol) to provide the title compound as a white solid (154 mg).

EXAMPLE 3

P-(1-Naphtlzyl)-L-alanine-L-leucine boronic acid hydrochloridesalt[MG-302] and β-(1-Naphthlyl)-L-alanine-L-leucine boronic acid [MG-303]

A. (1S,2S,3R,5S)-Pinanediol β-(1-naphthyl)-L-alanine-L-leucine boronate hydrochloride salt

To a solution of (1S,2S,3R,5S)-pinanediol β-(1-naphthyl) -L-alanine-L-leucine boronate trifluoroacetate salt (prepared

as described in Example 1B. 536 mg. 0.93 mmol) in ether (2 mL) was added 10 mL of 1N HCl. The mixture was sonicated for several minutes. Ether was allowed to slowly evaporate. The resultant crystals were collected. washed with H_2O and ether. and dried in vacuo to provide the title compound (300 mg).

B. β-(1-Naphthyl)-L-alanine-L-leucine boronic acid hydrochloride salt: and β-(1-Naphthyl)-L-alanine-L-leucine boronic acid

To the product of Example 3A (290 mg. 0.58 mmol) in a mixture of hexane (4 mL). MeOH (4 mL). and 1N HCl (1.3 mL) was added i-BuB(OH)$_2$ (71 mg. 0.70 mmol). The reaction mixture was stirred for 72 h at room temperature. The MeOH-H_2O layer was washed with hexanes. and the MeOH was evaporated. The aqueous solution was made basic with NaOH and washed with ether-EtOAc (1:1). The aqueous layer was lyophilized to give 640 mg of a yellow solid. The solid was dissolved in MeOH. 4N HCl in 1.4-dioxane was added. and the solution was filtered to remove a white solid. The filtrate was concentrated and the residue was purified by reverse phase HPLC (elution with CH_3CN—H_2O) to afford 45 mg of MG-302 and 10 mg of MG-303.

EXAMPLE 4

N-(4-Morphloline)carbonyl-(O-benzyl)-L-tyrosine-L-leucine boronic acid [MG-306]

A. N-Boc-O-Benzyl-L-tyrosine

A suspension of O-benzyl-L-tyrosine (3.12 g. 11.5 mmol) in a mixture of 1.4-dioxane (14 mL) and water (14 mL) was treated. in order. with triethylamine (5.0 mL. 35.9 mmol) and a solution of $(Boc)_2O$ (2.86 g. 13.1 mmol) in 1.4-dioxane (12 mL). After 19 h. the reaction mixture was diluted with water (140 mL) and washed with ether. The aqueous layer was acidified with 1N citric acid (35 mL) and extracted with CH_2Cl_2 (2×100 mL). Additional citric acid (15 mL) was added to the aqueous layer. which was again extracted with CH_2Cl_2 (100 mL). The combined organic extracts were dried ($MgSO_4$). filtered. and concentrated to give the crude product (4.5 g). which was used directly in the next reaction.

B. (1S.2S.3R.5S)-Pinanediol N-Boc-(O-benzyl)-L-tyrosine-L-leucine boronate

To a stirred and cold (0° C.) solution of (1S.2S.3R.5S)-pinanediol β-(1-naphthyl)-L-alanine-L-leucine boronate trifluoroacetate salt (prepared as described in Example 1B. 3.03 g. 7.98 mmol). N-Boc-O-benzyl-L-tyrosine (2.97 g. 7.99 mmol). and TBTU (3.35 g. 8.84 mmol) in anhydrous DMF (30 mL) was added by syringe pump. at the rate of 1.9 mL/h. DIEA (4.2 mL. 24.1 mmol). After the addition was complete. the mixture was allowed to warm to room temperature over 30 min. and then it was added dropwise to 30 mL of rapidly stirring water. Additional water was added and the mixture was filtered. The collected solid was dissolved in MeOH. concentrated to near dryness and again added to rapidly stirring water (300 mL). The resultant white solid was collected by suction filtration. washed with water. frozen. and lyophilized to provide the title compound (4.49 g).

C. (1S.2S.3R.5S)-Pinanediol (O-benzyl)-L-tyrosine-L-leucine boronate

The product of Example 4B (4.47 g. 7.23 mmol) was dissolved in CH_2Cl_2 (40 mL) and cooled to 0° C. A solution of 4N HCl in dioxane (40 mL. 0.16 mol) was added and the reaction mixture was stirred at room temperature for 1.5 h. Concentration afforded a yellow solid. which was triturated with hexane-ether (1:1. 100 mL). Filtration afforded the title compound (3.65 g) as a pale yellow solid.

D. (1S.2S.3R.5S)-Pinanediol N-(4-morpholine) carbonyl-(O-benzyl)-L-tyrosine-L-leucine boronate

By a procedure analogous to that described in Example 1C. the product of Example 4C (2.53 g. 4.56 mmol) was treated with 4-morpholinecarbonyl chloride (0.75 mL. 6.43 mmol) to provide the title compound (2.35 g) as a pale yellow solid.

E. N-(4-morpholine)carbonyl-(O-benzyl)-L-tyrosine-L-leucine boronic acid

The product of Example 4D (0.39 g. 0.62 mmol) was deprotected according to the procedure described in Example 3B to provide the title compound (146 mg) as a white solid.

EXAMPLE 5

N-Methyl-N-Cbz-L-leucine-L-leucine boronic acid [MG-268]

A. N-Methyl-N-Cbz-L-leucine

To a solution of N-Cbz-leucine (1.38 g. 5.2 mmol) in THF (15 mL) at 0° C. was added methyl iodide (2.5 mL. 40.1 mmol). Sodium hydride (60% dispersion in oil. 0.6 g. 15 mmol) was added cautiously. and the resultant mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with EtOAc (25 mL) and water (2 mL) was added dropwise. The mixture was concentrated to dryness. and the residue was partitioned between ether (15 mL) and water (50 mL). The organic layer was extracted with saturated aqueous $NaHCO_3$ (25 mL). and the combined aqueous extracts were acidified to pH 2 with 3N HCl. The product was extracted with EtOAc (3×25 mL). dried over $MgSO_4$. filtered. and concentrated to afford the title compound (1.41 g) as a yellow solid.

B. (1S.2S.3R.5S)-Pinanediol N-methyl-N-Cbz-L-leucine-L-leucine boronate

By a procedure analogous to that described in Example 1A. the product of Example 5A (85.1 mg. 0.30 mmol) was coupled with (1S.2S.3R.5S)-pinanediol leucine boronate trifluoroacetate salt (105 mg. 0.28 mmol) in the presence of EDC (64 mg. 0.33 mmol). HOBT (45 mg. 0.33 mmol). and NMM (37 mg. 0.37 mmol) to provide. after purification by flash chromatography (elution with 3:2 hexanes/acetone). the title compound (85 mg).

C. N-Methyl-N-Cbz-L-leucine-L-leucine boronic acid

By a procedure analogous to that described in Example 1D. the product of Example 5B (85 mg. 0.16 mmol) was deprotected by treatment with $NaIO_4$ (104 mg. 0.485 mmol) and aqueous NH_4OAc (0.1N. 5 mL. 0.5 mmol) in 10 mL of acetone to provide. after purification by flash chromatography (elution with 4:4:2 hexanes/acetone/MeOH). the title compound (21 mg).

EXAMPLE 6

N-(4-Morpholine)carbonyl-β-(6-quinolinyl)-D. L-alanine-L-leucine boronic acid [MG-292]

A. β-(6-Quinolinyl)-D.L-alanine

N-Acetyl β-(6-quinolinyl)-D.L-alanine ethyl ester (728 mg. 2.55 mmol) was heated at reflux in 6N HCl (20 mL).

After 20 h, the reaction mixture was concentrated to dryness and the residue was dried in vacuo to provide the title compound, which was used directly in the next reaction.

B. N-Boc-β-(6-Quinolinyl)-D,L-alanine

To the crude product of Example 6A in a stirred mixture of 1,4-dioxane (10 mL), water (10 mL), and 2N NaOH (5 mL) at 0° C. was added di-tert-butyl pyrocarbonate (556 mg, 2.55 mmol). The reaction mixture was allowed to warm to room temperature. After 23 h, the reaction mixture was acidified to pH 4 and extracted with EtOAc (3×50 mL) and n-BuOH (3×50 mL). The combined extracts were concentrated to provide the title compound, which was used directly in the next reaction.

C. (1S,2S,3R,5S)-Pinanediol N-Boc-β-(6-quinolinyl)-D, L-alanine-L-leucine boronate

By a procedure analogous to that described in Example 2A, the product of Example 6B was coupled with (1S,2S, 3R,5S)-pinanediol leucine boronate trifluoroacetate salt (943 mg, 2.5 mmol) in the presence of BOP reagent (1.33 g, 3 mmol) and triethylamine (0.37 mL, 2.62 mmol) to provide the title compound (343 mg).

D. (1S,2S,3R,5S)-Pinanediol β-(6-quinolinyl)-D, L-alanine-L-leucine boronate

The product of Example 6C (343 mg, 0.61 mmol) was treated with trifluoroacetic acid (7 mL) and thioanisole (1 mL) in CH_2Cl_2 (15 mL) at 0° C., as described in Example 1B, to provide the title compound.

E. (1S,2S,3R,5S)-Pinanediol N-(4-morpholine) carbonyl-β-(6-quinolinyl)-D,L-alanine-L-leucine boronate

The product of Example 6D was coupled with 4-morpholinecarbonyl chloride (0.14 mL, 1.22 mmol) by a procedure analogous to that described in Example 1C to produce the title compound (112 mg).

F. N-(4-Morpholine)carbonyl-β-(6-quinolinyl)-D, L-alanine-L-leucine boronate

Deprotection of the product of Example 6E (153 mg, 0.27 mmol) was effected according to the procedure described in Example 3B. Purification by silica gel chromatography (elution with 50:50:10 hexanes/acetone/methanol) afforded the title compound (87 mg). The product was further purified by reverse phase HPLC; 5 mg of the title compound was recovered.

EXAMPLE 7

N-(4-Morpholine)carbonyl-β-(1-naphthlzyl)-L-alanine-L-leucine methylboronic acid [MG-317]; and N-(4-Morpholine) carbonyl-β-(1-naphthyl)-L-alanine-L-leucine dimethylborane [MG-318]

To a suspension of MG-273 (prepared as described in Example 1, 101.5 mg, 0.23 mmol) in 3 mL of a 2:1 mixture of Et_2O/CH_2Cl_2 was added 1,3-propanediol (20.0 mL, 0.28 mmol). The resultant clear solution was stirred for 30 min at room temperature, and then anhydrous $MgSO_4$ was added. Stirring was continued for an additional 30 min, and then the mixture was filtered through a cotton plug and then through a 0.2 mm PTFE filter. The solution was concentrated, toluene (2 mL) was added, and the mixture was again concentrated to produce a white solid. Anhydrous THF (3 mL) was added, and the resultant solution was cooled to 0° C. MeLi (0.8 mL, 1.12 mmol) was added. After 10 min, the mixture was warmed to room temperature. After 20 min, the light red solution was cooled to 0° C., quenched with a few drops of water, and then diluted with 10 mL of 1N HCl. The colorless solution was extracted with CH_2Cl_2 (2×10 mL), and the combined extract was concentrated to afford a white solid. Purification by flash chromatography (elution with 2–4% MeOH/$CHCl_3$, followed by 10% MeOH/$CHCl_3$) afforded MG-317 (17.7 mg) and MG-318 (72.1 mg).

EXAMPLE 8

N-Benzyl-(3R)-3-dioxyboryl-5-metlzylliexanamide [MG-342]

A. tert-Butyl-(3R)-3-[(1S,2S,3R,5S)-(pinanediyldioxy)boryl]-5-metliylliexanoate

A 200-mL round-bottomed flask was charged with anhydrous THF (50 mL) and tert-butyl acetate (0.48 mL, 3.56 mmol). The solution was cooled to −78° C. under nitrogen, and LDA (1.5M solution in cyclohexane, 2.2 mL, 3.3 mmol) was added by syringe over 8 min. The resultant solution was stirred for 10 min, and then a solution of (1S,2S,3R,5S)-pinanediol 1-bromo-3-methylbutylboronate (*Organometallics* 9:3171 (1990)) (1.04 g, 3.15 mmol) in anhydrous THF (15 mL) was added by cannula over 8 min. The reaction mixture was allowed to warm to room temperature and stir overnight. The pale pink solution was concentrated, and the residue was dissolved in 200 mL of ether. The solution was washed with saturated aqueous NH_4Cl and saturated aqueous NaCl. Concentration gave a clear orange oil, which was purified by flash chromatography (elution with 2–3% EtOAc/hexanes) to afford the title compound (584 mg).

B. (3R)-3-[(1S,2S,3R,5S)-(pinanediyldioxy)boryl]-5-metlhylhexanoic acid

To a solution of the product of Example 8A (323 mg, 0.89 mmol) in CH_2Cl_2 (8 mL) was added trifluoroacetic acid (2.0 mL, 26 mmol). The resultant mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated and dried overnight under high vacuum to produce a dark brown oil (309.3 mg).

C. N-Benzyl-(3R)-3-[(1S,2S,3R,5S)-pinanediyldioxy)boryl]-5-metliylliexanamide

To a solution of the product of Example 8B (300 mg, 0.9 mmol) and TBTU (410 mg, 1.08 mmol) in anhydrous acetonitrile (5 mL) was added benzylamine (0.12 mL, 1.10 mmol), followed by diisopropylethylamine (0.50 mL, 2.9 mmol). The reaction mixture was stirred overnight at room temperature, and then was poured into water and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl. Concentration gave a dark brown oil, which was purified by flash chromatography (elution with 20% EtOAc/hexanes) to afford the title compound (232 mg) as a clear, colorless oil.

D. N-Benzyl-(3R)-3-dioxyboryl-5-metliylliexanamide

The product of Example 8C (223 mg, 0.56 mmol) was deprotected according to the procedure described in Example 3B. Purification by flash chromatography (elution with 5% MeOH/$CHCl_3$) provided a pale yellow oil, which was dissolved in acetonitrile/MeOH. Water was added and

the mixture was lyophilized overnight to produce the title compound (108 mg) as a fluffy white solid.

EXAMPLE 9

N-Acetyl-1,2,3,4-tetrahydro-3-isoquinolinecarbonyl-L-leucine boronic acid [MG-310]

A. N-Boc-1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic acid

A solution of 1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (855 mg, 4.83 mmol), $(Boc)_2O$ (1.37 g, 6.28 mmol), and 1N NaOH (6 mL) in a mixture of t-BuOH (12 mL) and water (12 mL) was stirred overnight at room temperature. The reaction mixture was diluted with water (30 mL) and washed with ether-hexanes (1:1,2×25 mL). The organic layer was back-extracted with 10% $NaHCO_3$. The combined aqueous layers were carefully acidified to pH 2–3 and extracted with EtOAc (3×30 mL). The combined organic extracts were washed with water and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated to provide the title compound (1.27 g) as a white solid.

B. (1S,2S,3R,5S)-Pinanediol N-Boc-1,2,3,4-tetrahydro-3-isoquinolinecarbonyl-L-leucine boronate

To a mixture of (1S,2S,3R,5S)-pinanediol-L-leucine boronate trifluoroacetate salt (1.14 g, 3.03 mmol), N-Boc-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (762 mg, 2.75 mmol), and BOP reagent (1.34 g, 3.03 mmol) in DMF (20 mL) was added, over a period of 2 h, DIEA (1.44 mL, 8.25 mmol). The resultant solution was stirred for 1 h after addition was complete. The reaction mixture was poured into water (300 mL) and extracted with EtOAc (3×75 mL). The combined organic extracts were washed with dilute aqueous HCl, half-saturated aqueous $NaHCO_3$, water, and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (elution with 20% EtOAc-hexanes) to provide the title compound (1.04 g) as a white foamy solid.

C. (1S,2S,3R,5S)-Pinanediol 1,2,3,4-tetrahydro-3-isoquinolinecarbonyl-L-leucine boronate hydrochloride salt

The product of Example 9B (755 mg) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0° C. A solution of 4N HCl in dioxane (8 mL, 0.03 mol) was added and the reaction mixture was stirred at room temperature. Concentration and trituration with ether-hexanes afforded the title compound (565 mg) as an off-white solid.

D. (1S,2S,3R,5S)-Pinanediol N-acetyl-1,2,3,4-tetrahydro-3-isoquinolinecarbonyl-L-leucine boronate

The product of Example 9C (262 mg, 0.59 mmol) was treated at room temperature with Ac_2O (0.085 mL, 0.89 mmol) and DIEA (0.18 mL, 1.36 mmol) in CH_2Cl_2 (5 mL). After 24 h, the reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with 1N HCl, half-saturated $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. Purification by flash chromatography (elution with EtOAc-hexanes) afforded the title compound (271 mg) as a white foamy solid.

E. N-Acetyl-1,2,3,4-tetrahydro-3-isoquinolinecarbonyl-L-leucine boronic acid

By a procedure analogous to that described in Example 3B, the product of Example 9D (226 mg, 0.49 mmol) was deprotected to provide the title compound (131 mg) as a foamy, oily solid.

EXAMPLE 10

N-(4-Morpholine)carbonyl-β-(2-quinolyl)-L-alanine-L-leucine boronic acid [MG-315]

A. Diethyl (2-quinolylmethyl)acetamidomalonate

To a solution of 2(chloromethyl)quinoline monohydrochloride (5.0 g, 23.4 mmol) and diethyl acetamidomalonate (10.1 g, 46.7 mmol) in EtOH (60 mL) was added sodium methoxide (3.78 g, 70 mmol). The reaction mixture was heated at reflux for 6 h. The reaction mixture was cooled, filtered, and concentrated. The residue was dissolved in EtOAc (400 mL) and extracted with cold 4N HCl (3×150 mL). The aqueous layer was neutralized with 10N NaOH and extracted with EtOAc (3×200 mL). The combined organic extract was washed with water, dried (anhydrous $MgSO_4$), filtered, and concentrated to give the title compound (8.3 g).

B. N-Acetyl-β-(2-quinolyl)-D,L-alanine ethyl ester

To a solution of the product of Example 10A (8 g, 22.3 mmol) in EtOH (180 mL) was added 6.1N NaOH (6.5 mL, 40 mmol). After 2 h, 11.1N HCl (3.6 mL, 40 mmol) was added, and the reaction mixture was concentrated to dryness. The residue was suspended in 1,4-dioxane (200 mL) and the mixture was heated at reflux for 90 min. The reaction mixture was concentrated and the residue was purified by silica gel chromatography (elution with 30–50% acetone-hexanes) to provide to title compound (4.3 g).

C. N-Acetyl-β-(2-quinolyl)-L-alanine

The product of Example 10B (4.3 g, 15 mmol) was treated with Subtilisin Carlsberg (Sigma, 11.9 units/mg, 30 mg, 357 units) at room temperature in aqueous $NaHCO_3$ (0.2M, 120 mL). After 2 h, the reaction mixture was extracted with $CHCl_3$ (6×100 mL). The aqueous layer was concentrated to dryness to provide the title compound (3.5 g), which contained salts.

D. N-Boc-β-(2-Quinolyl)-L-alanine

A solution of the product of Example 10C (3.5 g, ca. 7.4 mmol) in 6N HCl (40 mL) was heated at reflux for 16 h. The solvent was removed and the residue was dried in vacuo.

To this residue was added 1,4-dioxane (20 mL), water (20 mL), and 2N NaOH (10 mL, 20 mmol). The solution was cooled to 0° C. and di-t-butyl pyrocarbonate (1.6 g, 7.5 mmol) was added. After 1 h at 0° C., the reaction mixture was warmed to room temperature and stirring was continued for 17 h. The reaction mixture was extracted with CH_2Cl_2 (100 mL) and n-BuOH (4×100 mL). The aqueous layer was acidified and again extracted with n-BuOH. The organic extracts were combined and concentrated to provide the title compound (1.6 g).

E. (1S,2S,3R,5S)-Pinanediol N-Boc-β-(2-quinolyl)-L-alanine-L-leucine boronate

By a procedure analogous to that described in Example 2A, the product of Example 10D (0.6 g, 1.9 mmol) was coupled with (1S,2S,3R,5S)-pinanediol leucine boronate trifluoroacetate salt (716 mg, 1.9 mmol) in the presence of BOP reagent (0.84 g, 1.9 mmol) and triethylamine (0.27 mL, 1.9 mmol). Purification by silica gel chromatography

(elution with 10–30% acetone-hexanes) afforded the title compound (194 mg).

F. (1S,2S,3R,5S)-Pinanediol N-(4-morpholine) carbonyl-β-(2-quinolyl)-L-alanine-L-leucine boronate

The product of Example 10E (194 mg) was treated with trifluoroacetic acid (7 mL) and thioanisole (1 mL) as described in Example 1B. The resultant product was condensed with 4-morpholinecarbonyl chloride (568 mg, 3.8 mmol) as described in Example 2C. Purification by silica gel chromatography (elution with 20–50% acetone-hexanes) afforded the title compound (367 mg).

G. N-(4-Morpholine)carbonyl-β-(2-quinolyl)-L-alanine-L-leucine boronic acid

The product of Example 10F (367 mg, 0.64 mmol) was deprotected according to the procedure described in Example 3B to provide the title compound (222 mg).

EXAMPLE 11

N-Boc-1,2,3,4-tetrahydro-1-isoquinolinecarboxylic acid [precursor for the synthesis of MG-310]

A. 1,2,3,4-Tetrahydro-1-isoquinolinecarboxylic acid

A solution of 1-isoquinolinecarboxylic acid (1.67 g) in glacial acetic acid (25 mL) was hydrogenated at 60 p.s.i. over PtO_2 (270 mg). When the reaction was complete, the mixture was filtered through diatomaceous earth (Celite), washing the solid pad with MeOH, and the filtrate was concentrated to dryness. The resultant white solid was triturated with cold water and filtered to provide the title compound (775 mg).

B. N-Boc-1,2,3,4tetrahydro-1-isoquinolinecarboxylic acid

The product of Example 11B (762 mg, 4.3 mmol) was treated with di-tert-butyl pyrocarbonate (1.13 g, 5.17 mmol) according to the procedure described in Example 6B to afford the title compound (886 mg), as a foamy white solid.

EXAMPLE 12

Diethanolamine N-(4-morpholine)carbonyl-β-(1-naphtilyl)-L-alanine-L-leucine boronate [MG-286]

To a solution of N-(4-morpholine)carbonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid (prepared as described in Example 1, 97.4 mg, 0.22 mmol) in CH_2Cl_2 (4 mL) was added a solution of diethanolamine (25.5 mg, 0.24 mmol) in EtOAc (1 mL). The resultant solution was stirred at room temperature for 0.5 h. Anhydrous Na_2SO_4 (1.5 g) was added and stirring was continued for an additional 0.5 h. The reaction mixture was filtered and concentrated, and the crude product was purified by stirring in hot EtOAc (2 mL) and precipitation with hexanes (1 mL). The solid was collected, washed with hexanes, and dried to provide the title compound (106 mg).

EXAMPLE 13

N-[3-(4-morpholine)carbonyl-2(R)-(1-naphthyl) methyl] propionyl-L-leucine boronic acid [MG-324]

A. 1-naphthalenecarboxaldehyde

To a cold (−78° C.) solution of oxalyl chloride (6.9 mL, 0.079 mol) in dry CH_2Cl_2 (200 mL) was added dropwise dry DMSO (11.2 mL, 0.158 mol). The mixture was stirred for 10 min. and then a solution of 1-naphthalenemethanol (10.0 g, 0.063 mol) in dry CH_2Cl_2 (40 mL) was added over 15 min. The mixture was stirred for 10 min. and then Et_3N (44 mL, 0.316 mol) was added slowly. The reaction mixture was allowed to warm to room temperature. After 3.5 h, to the pale yellow heterogeneous mixture was added 10% aqueous citric acid (30 mL) and water (100 mL). The organic phase was washed with water (100 mL) and saturated aqueous NaCl (100 mL), dried (anhydrous $MgSO_4$), filtered, and concentrated. Ether-hexane (1:1) was added and the mixture was filtered. Concentration provided a pale orange oil (9.7 g).

B. Ethyl 3-(1-naphthyl)propionate

To a solution of the product of Example 12A (9.7 g, 62 mmol) in CH_2Cl_2 (150 mL) was added at room temperature (carbethoxymethylene) triphenylphosphorane (25 g, 71 mmol). The resultant mixture was stirred for 1.5 h, and the homogeneous yellow solution was then concentrated to dryness. Ether-hexane (1:1) was added, the mixture was filtered, and the filtrate was concentrated to dryness to provide a pale orange oil (15.3 g).

C. Ethyl 3-(1-naphthyl)propionate

The product of Example 12B (15.3 g, 68 mmol) was dissolved in a mixture of EtOAc (100 mL) and MeOH (10 mL) and hydrogenated at 1 atm. over 10% Pd/C (0.5 g). The reaction was continued for 4 days, replacing the catalyst with fresh catalyst several times. The reaction mixture was filtered and concentrated to provide 13 g of a crude oil.

D. 3-(1-Naphthyl)propionic acid

To a solution of the product of Example 12C (13 g) in a mixture of THF (100 mL) and water (25 mL) was added 1N NaOH (75 mL, 75 mmol). The brown reaction mixture was stirred at room temperature overnight. The THF was removed, and the aqueous layer was washed with ether (2×50 mL). The aqueous layer was acidified to pH 2 with 6N HCl and the precipitated solid was collected, washed with water (100 mL), and lyophilized to give 9.3 g of a pale yellow solid.

E. 3-(1-Naphtliyl)propionyl chloride

To a suspension of the product of Example 12D (4.0 g, 20 mmol) in CH_2Cl_2 (25 mL) at 0° C. was added oxalyl chloride (1.9 mL, 22 mmol) and DMF (0.1 mL). The reaction mixture was warmed to room temperature and then heated with a heat gun. Additional oxalyl chloride (0.5 mL) was added and heating was continued to produce a dark homogeneous mixture. The reaction mixture was concentrated, the residue was redissolved in CH_2Cl_2-hexane, and the resultant solution was filtered. Concentration afforded 4.9 g of a green liquid.

F. 4(S)-Isopropyl-3-[3-(1-naphthyl)-1-oxopropyl]-2-oxazolidinone

To a solution of (4S)-(-)-4-isopropyl-2-oxazolidinone (2.32 g, 18 mmol) in dry THF (50 mL) at −78° C. was added dropwise n-BuLi (2.5M in hexanes, 8 mL, 20 mmol). The heterogeneous white mixture was stirred at −78° C. for 30 min. and then a solution of the product of Example 12E (4.9 g, 20 mmol) in dry THF (25 mL) was added dropwise over 15–20 min. After 1.5 h, the reaction was quenched by the addition of 1N HCl (25 mL) and saturated aqueous NaCl (25

mL). The mixture was stirred at room temperature for 30 min. and then the THF was removed by rotary evaporation. The aqueous layer was extracted with EtOAc. and the combined organic extract was dried (anhydrous $MgSO_4$). filtered. and concentrated. The residue was filtered through a pad of silica gel (elution with 20% EtOAc-hexanes) to provide 2.8 g of a pale pink solid.

G. 3-[3-Benzyloxycarbonyl-2(R)-[(1-naphthyl) methyl]-1-oxopropyl]-4(S)-isopropyl-2-oxazolidinone

To a solution of 1.1.1.3.3.3-hexamethyldisilazane (0.75 mL. 3.5 mmol) in dry THF (10 mL) at 0° C. was added n-BuLi (2.5M in hexanes. 1.45 mL. 3.6 mmol). After 10 min. the mixture was cooled to −78° C. and a solution of the product of Example 12F (1.0 g. 3.2 mmol) in dry THF (8 mL) was added dropwise. After 30–40 min. benzyl bromoacetate (0.75 mL. 4.8 mmol) was added. The mixture was stirred at −78° C. for 1 h. and at 0° C. for 5–10 min. The reaction was quenched by the addition of 1N HCl (10 mL). and the solution was extracted with ether. The combined organic extract was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl. dried anhydrous $MgSO_4$). filtered and concentrated. The wet solid was triturated with hexane-ether (1:1). filtered. and dried to give the title compound (0.6 g) as a white solid.

H. 3-[2(R)-(1-naphthyl)methyl]-3-[4(S)-isopropyl-2-oxazolidinoyl]propanoic acid

To the product of Example 12G (600 mg. 1.3 mmol) was added MeOH (15 mL). EtOH (15 mL). EtOAc (5 mL). and CH_2Cl_2 (5 mL). followed by 10% Pd/C (100 mg). The reaction mixture was hydrogenated under 1 atm. H_2. The reaction mixture was filtered and concentrated. The residue was triturated with ether-hexanes. the solvents were removed. and the resultant white solid was dried in vacuo to give 480 mg of the title compound.

I. 4(S)-Isopropyl-3-[4-morpholino-2(R)-(1-naphthyl) methyl-1.4-dioxobutyl]-2-oxazolidinone

To a solution of the product of Example 12H (473 mg. 1.28 mmol) in dry THF (25 mL) at 0° C. was added dropwise under nitrogen morpholine (130 mL. 1.47 mmol). diethyl pyrocarbonate (240 mL. 1.47 mmol). and triethylamine (220 mL. 1.6 mmol). After 2 h. the solvent was removed in vacuo. and the residue was washed with water and extracted with ether-EtOAc (1:1). The combined organic extract was dried (anhydrous $MgSO_4$). filtered. and concentrated. The residue was triturated with EtOAc-hexanes to provide the title compound (410 mg).

J. 3-(4-morpholine)carbonyl-2(R)-(1-naphthyl) methyl propionic acid

To a solution of the product of Example 12I (400 mg. 0.913 mmol) in a mixture of THF (8 mL) and water (2 mL) at 0° C. was added LiOH (80 mg. 1.9 mmol). The reaction mixture was stored at 0° C. overnight. The reaction mixture was concentrated to remove THF. 1N NaOH (20 mL) was added. and the mixture was washed with CH_2Cl_2 (15 mL). The aqueous layer was acidified to pH 2 with 1N HCl and extracted with CH_2Cl_2. The combined organic extract was dried (anhydrous $MgSO_4$). filtered. and concentrated. The residue was triturated with ether-hexanes. and the solvents were removed in vacuo to provide the crude product (240 mg) as a white foam.

K. (1S.2S.3R.5S)-Pinanediol N-[3-(4-morpholine) carbonyl-2(R)-(1-naphthyl)methyl]propionyl-L-leucine boronate

To a solution of the product of Example 12J (230 mg. 0.7 mmol) in DMF (8 mL) at 0° C. was added (1S.2S.3R.5S)-pinanediol leucine boronate trifluoroacetate salt (293 mg. 0.77 mmol) and TBTU (293 mg. (0.77 mmol). To the resultant mixture was added slowly over 1.5 h diisopropylethylamine (365 mL. 2.1 mmol). After addition was complete. the reaction mixture was stirred for 30 min. Water (100 mL) was added. and the precipitated solid was collected. washed with water (50 mL). and lyophilized to provide the title compound (300 mg).

L. N-[3-(4-morpholine)carbonyl-2(R)-(1-naphthyl) methyl]propionyl-L-leucine boronic acid

By a procedure analogous to that described in Example 3B. the product of Example 12K (300 mg. 0.522 mmol) was deprotected to provide the title compound (150 mg).

EXAMPLE 14

trans-4-Phenoxy-L-proline-L-leucine boronic acid [MG-349]

A. N-Carbobenzyloxy-trans-4-hydroxy-L-proline

According to the literature procedure (*J. Am. Chem. Soc.* 189 (1957)). trans-4-hydroxy-L-proline (5.12 g. 0.039 mol) was treated with benzyl chloroformate (8.5 mL. 0.06 mol) to provide the title compound (6.0 g) as a white solid.

B. N-Carbobenzyloxy-trans-4-hydroxy-L-proline methyl ester

To a solution of the product of Example 13A (1.08 g. 3.75 mmol) in acetonitrile (4 mL) at 0° C. was added dropwise DBU (0.62 mL. 4.12 mmol). After 5 min. MeI (0.28 mL. 4.5 mmol) was added. The reaction mixture was allowed to warm to room temperature and stir overnight. The solvent was removed. the residue was dissolved in ether-EtOAc (1:1. 30 mL). and the resultant solution was washed with 1N HCl. dilute aqueous $NaHCO_3$. water. and saturated aqueous NaCl. The organic layer was dried (anhydrous $MgSO_4$) and concentrated to provide the title compound (822 mg) as a light yellow oil.

C. N-Carbobenzyloxy-trans-4-phenoxy-L-proline methyl ester

To a mixture of the product of Example 13B (495 mg. 1.71 mmol). phenol (193 mg. 2.05 mmol). and triphenylphosphine (537 mg. 2.05 mmol) in THF (7 mL) at 0° C. was added over 1 h diethyl azodicarboxylate (0.32 mL. 2.05 mmol). The reaction mixture was allowed to warm to room temperature and stir overnight. The reaction mixture was concentrated. and the residue was dissolved in ether (8 mL) and allowed to stand at 0° C. overnight. The solution was decanted and the solids were washed with cold ether. The ethereal solution was concentrated. and the residue was purified by flash chromatography (elution with 10–30% EtOAc-hexanes) to provide the title compound (295 mg).

D. N-Carbobenzyloxy-trans-4-phenoxy-L-proline

The product of Example 13C (285 mg. 0.79 mmol) was dissolved in a mixture of 0.5N aqueous LiOH (20 mL) and MeOH (10 mL). and the resultant solution was stirred at room temperature overnight. The MeOH was removed in

vacuo. and the aqueous layer was washed with ether (2×20 mL). The aqueous layer was cooled. acidified with 3N HCl. and extracted with EtOAc (3×20 mL). The combined organic extract was washed with water and saturated aqueous NaCl. dried (anhydrous $MgSO_4$). filtered. and concentrated to provide the title compound (251 mg) as a light yellow solid.

E. (1S.2S.3R.5S)-pinanediol N-Carbobenzyloxy-trans-4-phenoxy-L-proline-L-leucine boronate

By a procedure analogous to that described in Example 12K. the product of Example 13D (250 mg. 0.72 mmol) was coupled with (1S.2S.3R.5S)-pinanediol leucine boronate trifluoroacetate salt (300 mg. 0.79 mmol) in the presence of TBTU (302 mg. 0.79 mmol) to provide the title compound (355 mg) as a white solid.

F. (1S.2S.3R.5S)-pinanediol trans-4-phenoxy-L-proline-L-leucine boronate

The product of Example 13E (343 mg) was hydrogenated for 20 h at 1 atm. over 10% Pd/C (45 mg) in EtOH (3 mL). The reaction mixture was filtered through Celite and concentrated to provide the title compound (272 mg).

G. trans-4-Phenoxy-L-proline-L-leucine boronic acid

By a procedure analogous to that described in Example 3B. the product of Example 13F (270 mg. 0.6 mmol) was deprotected to provide the title compound (130 mg) as a white solid.

EXAMPLE 15

[(3S.5R)-4-[(8-quinolinesulfonyl)amino]-3-4hydroxy-5-(1-naphthyl)pentanoyl]-L-leucine boronic acid

A. (4S.5S)-1-Boc-4-hydroxy-5-(1-naphthyl)-pyrrolidin-2-one

To a solution of N-Boc-β-(1-naphthyl)-L-alanine (1.4 g. 4.44 mmol). 2.2-dimethyl-1.3-dioxane-4.6-dione (704 mg. 4.88 mmol). and 4-DMAP (1.25 g. 10.21 mmol) in CH_2Cl_2 (40 mL) at 0° C. was added isopropenyl chloroformate (0.53 mL. 4.8 mmol). The reaction mixture was stirred for 1 h at 0° C. and for 2 h at room temperature. The reaction was quenched by the addition of aqueous $KHSO_4$. The organic layer was washed with water. dried (anhydrous $MgSO_4$). filtered. and concentrated. The residue was suspended in EtOAc (30 mL) and heated at reflux for 2 h. The solvent was removed in vacuo.

The residue was dissolved in CH_2Cl_2-HOAc (10:1. 30 mL). and sodium borohydride (310 mg. 8.21 mmol) was added at 0 ° C. The mixture was stirred for 1 h at 0° C. and for 15 h at room temperature. Water was added. and the organic layer was washed with saturated aqueous NaCl. dried (anhydrous $MgSO_4$). filtered. and concentrated. Purification by silica gel chromatography (elution with 20–30% acetone-hexanes) afforded the title compound (1.24 g).

B. (3S.5R)-4-(tert-butyloxycarbonyl)amino-3-hydroxy-5-(1-naphthyl)pentanoic acid

The product of Example 14B (1.24 g. 3.64 mmol) was dissolved in acetone (15 mL) and aqueous NaOH (1M. 4 mL. 4 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. The mixture was acidified with 10% HCl and extracted with EtOAc (3×60 mL). The combined organic extract was washed with water. dried (anhydrous $MgSO_4$). filtered. and concentrated. The residue was purified by silica gel chromatography (elution with 30–50% acetone-hexanes and 70:30: 10 hexane:acetone:methanol) to give the title compound (0.61 g).

C. (1S.2S.3R.5S)-Pinanediol[(3S.5R)-4-(tert-butyloxycarbonyl) amino-3-hydroxy-5-(1-naphthyl) pentanoyl]-L-leucine boronate

By a procedure analogous to that described in Example 2. the product of Example 14B (395 mg. 1.1 mmol) was coupled with (1S.2S.3R.5S)-pinanediol leucine boronate trifluoroacetate salt (415 mg. 1.1 mol) in the presence of BOP reagent (487 mg. 1.1 mmol) to afford the title compound (261 mg).

D. (1S.2S.3R.5S)-Pinanediol [(3S.5R)-4-(8-quinolinesulfonyl) amino-3-hydroxy-5-(1-naphthyl) pentanoyl]-L-leucine boronate

The product of Example 14C (261 mg. 0.43 mmol) was dissolved in CH_2Cl_2 (10 mL) and treated at 0° C. with trifluoroacetic acid (5 mL) and thioanisole (1 mL). After 2 h. solvents were evaporated.

The residue was dissolved in CH_2Cl_2 (10 mL) and cooled to 0° C. 8-Quinolinesulfonyl chloride (98 mg. 0.43 mmol) and triethylamine (0.12 mL. 0.86 mmol) were added. The reaction mixture was stirred at 0° C. for 1 h and at room temperature for 15 h. The solvents were removed. water was added. and the product was extracted with EtOAc (3×50 mL). The combined organic extract was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl. dried (anhydrous $MgSO_4$). and concentrated. The residue was purified by silica gel chromatography (elution with 20–50% EtOAc-hexanes) to provide the title compound (152 mg).

E. [(3S. 5R)-4-(8-quinolinesulfonyl)amino-3-hydroxy-5-(1-naphthyl)pentanoyl]-L-leucine boronic acid

The product of Example 14D (152 mg. 0.22 mmol) was deprotected according to the procedure described in Example 3B to provide the title compound (12.7 mg).

EXAMPLE 16

cis-3-Phenyl-D.L-proline-L-leucine boronic acid hydrochloride salt [MG-359]

A. Diethyl 1-acetyl-4-phenyl-2-pyrrolidinol-5.5-dicarboxylate

Sodium spheres (washed 3 x with hexanes and dried in vacuo; 0.13 g. 5.7 mmol) were added to a solution of diethyl acetimidomalonate (12.2 g. 56.1 mmol) in absolute EtOH under nitrogen. After the sodium had dissolved. the solution was cooled in an ice bath and cinnamaldehyde (7.8 mL. 61.7 mmol) was added dropwise. The bath was removed and the reaction mixture was stirred overnight at room temperature. The solution was adjusted to pH 4 with acetic acid (~3 mL). Solvents were evaporated and the residue was purified by silica gel chromatography (elution with EtOAc) to give a yellow solid. which was recrystallized (benzene-hexane) to provide the title compound (14.1 g) as a white solid.

B. Diethyl 1-acetyl-3-phenylpyrrolidine-2.2-dicarboxylate

Trifluoroacetic acid (15.4 mL) was added slowly over 15 min to a solution of the product of Example 15A (7.0 g. 20.1

mmol) and triethylsilane (4.9 mL, 30.8 mmol) in $CHCl_3$ (40 mL). After 3 h, the solvents were evaporated and the residue was dissolved in EtOAc (150 mL), washed with water, 5% aqueous $NaHCO_3$, and saturated aqueous NaCl, dried (anhydrous $MgSO_4$), and concentrated to give 5.9 g of a colorless oil.

C. N-Acetyl-3-phenylproline ethyl ester

The product of Example 15B (5.9 g) was dissolved in 0.5N NaOH (200 mL) and the resultant solution was stirred at room temperature for 21 h. The solution was washed with EtOAc (75 mL) and then acidified to pH 2 with 3N HCl. The precipitated solids were extracted with $CHCl_3$. The organic layer was concentrated to give a gummy residue, which was dissolved in toluene (70 mL) and heated at 75° C. for 1 h. The solvent was evaporated to provide the title compound (4.2 g) as a light yellow oil.

D. N-Acetyl-trans-3-phenyl-D,L-proline; and N-acetyl-cis-3-phenyl-D,L-proline ethyl ester

The product of Example 15C (4.2 g, 16 mmol) was dissolved 1M NaOEt in EtOH (100 mL) which contained 2 mL of ethyl trifluoroacetate as a water scavenger, and the resultant solution was heated at reflux for 2 h. The reaction mixture was cooled to room temperature, water (65 mL) was added, and the solution was stirred for 2.5 h. Most of the EtOH was removed by rotary evaporation and the aqueous solution was extracted with CH_2Cl_2. The aqueous layer was acidified with 3N HCl and extracted with EtOAc. The organic extract was washed with water and saturated aqueous NaCl, dried (anhydrous $MgSO_4$), and concentrated. The orange gummy solid was triturated with ether to provide a yellow solid, which was recrystallized (EtOAc-MeOH) to provide the acid (1.91 g) as light yellow crystals. Concentration of the CH_2Cl_2 extracts afforded the ester (396 mg) as an orange oil.

E. cis-3-Phenyl-D,L-proline hydrochloride salt

The ester obtained in Example 15D (375 mg) was hydrolyzed by heating at reflux in 6N HCl (5 mL) for 17 h. The cooled reaction mixture was washed with EtOAc and the aqueous layer was concentrated to dryness. Recrystallization (MeOH-ether) afforded the title compound (201 mg).

F. N-Boc-cis-3-Phenyl-D,L-proline

The product of Example 15E (189 mg, 0.84 mmol) was dissolved in a mixture of 2N NaOH (3 mL) and 1,4-dioxane (3 mL). tert-Butyl pyrocarbonate (218 mg, 1.0 mmol) was added and the reaction mixture was stirred overnight at room temperature. Dioxane was removed by rotary evaporation, water (30 mL) was added, and the mixture was washed with EtOAc. The aqueous phase was cooled to 0° C., acidified with 3N HCl, and extracted with EtOAc. The organic layer was washed with water and saturated aqueous NaCl, dried (anhydrous $MgSO_4$), and concentrated to give the title compound (199 mg).

G. (1S,2S,3R,5S)-Pinanediol N-Boc-cis-3-phenyl-D,L-proline-L-leucine boronate

By a procedure analogous to that described in Example 4B, the product of Example 15F (192 mg, 0.66 mmol) was coupled with (1S,2S,3R,5S)pinanediol leucine boronate trifluoroacetate salt (274 mg, 0.73 mmol) in the presence of TBTU (277 mg, 0.73 mmol) to provide the title compound (286 mg).

H. cis-3-Phenyl-D,L-proline-L-leucine boronic acid hydrochloride salt

The product of Example 15G (262 mg) was dissolved in CH_2Cl_2 (5 mL) and treated at 0° C. with 4N HCl-dioxane (4 mL). After 2 h, the reaction mixture was concentrated to dryness, and the residue was treated with isobutylboronic acid (66 mg, 0.64 mmol) according to the procedure described in Example 3B to provide the title compound (71 mg) as a white solid.

EXAMPLE 17

trans-3-Phenyl-D,L-proline-L-leucine boronic acid hydrochloride salt [MG-363]

A. N-Boc-trans-3-Phenyl-L-proline

By a procedure analogous to that described in Example 1A, N-acetyl-trans-3-phenyl-D,L-proline (prepared as described in Example 15D; 1.5 g, 6.44 mmol) was coupled with (S)-a-methylbenzylamine (0.92 mL, 7.08 mmol) in the presence of EDC (1.26 g, 7.08 mmol) and HOBT 9956 mg, 7.08 mmol). The diastereomeric products were separated by flash chromatography (elution with 1.5–2.5% HOAc-EtOAc). Fractions corresponding to the slower eluting band were concentrated to provide a clear, colorless oil (913 mg).

The oil (900 mg, 2.68 mmol) was dissolved in a mixture of HOAc (7 mL) and 8N HCl and the mixture was heated at reflux for 18 h. The mixture was concentrated to dryness. The residue was dissolved in water (30 mL), washed with EtOAc, and again concentrated to dryness.

The residue was redissolved in 1:1 water-1,4-dioxane (15 mL) and treated with tert-butyl pyrocarbonate (1.13 g, 5.20 mmol) by a procedure analogous to that described in Example 15F to provide the title compound (574 mg) as a white solid.

B. trans-3-Phenyl-L-proline-L-leucine boronic acid hydrochloride salt

By procedures analogous to those described in Examples 15G–H, the product of Example 16A (332 mg, 1.14 mmol) was coupled with (1S,2S,3R,5S)-pinanediol leucine boronate trifluoroacetate salt (452 mg, 1.20 mmol) and deprotected to provide the title compound (101 mg) as a white solid.

EXAMPLE 18

Kinetic experiments

Table II summarizes results from kinetic experiments that measured the inhibition of the 20S proteasome by compounds having the formula of compound (1) or (2). P, AA^1, AA^2, AA^3, and Z^1 and Z^2 represent the structures present on formula (1) or (2). The protocol for the kinetic assay described in Tables II–V is as described in Rock et al., *Cell* 78,761–771 (1994). In these tables, K_i values are reported, which are dissociation constants for the equilibrium that is established when enzyme and inhibitor interact to form the enzyme:inhibitor complex. The reactions were performed using SDS-activated 20S proteasome from rabbit muscle. The substrate used was Suc-LLVY-AMC.

TABLE II

Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
P – AA^1 – AA^2 – AA^3 – $B(Z^1)(Z^2)$

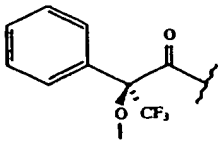
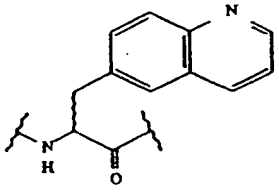
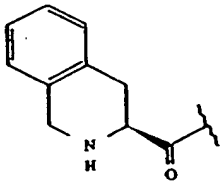
Compound	P[a]	AA^1	AA^{2b}	AA^{3c}	Z^1, Z^2	20S K_i (nM)
MG-261	Cbz	L–Leu	L–Leu	L–Leu	pinane diol	0.032
MG-262	Cbz	L–Leu	L–Leu	L–Leu	$(OH)_2$	0.035
MG-264	Cbz	—	L–Leu	L–Leu	pinane diol	119.00
MG-267	Cbz	—	L–Nal	L–Leu	pinane diol	0.100
MG-268	Cbz(N–Me)	—	L–Leu	L–Leu	$(OH)_2$	998.00
MG-270	Cbz	—	L–Nal	L–Leu	$(OH)_2$	0.083
MG-272	Cbz	—	D-(2-Nal)	L–Leu	$(OH)_2$	34.0
MG-273	Morph	—	L–Nal	L–Leu	$(OH)_2$	0.18
MG-274	Cbz	—	L–Leu	L–Leu	$(OH)_2$	3.0
MG-278	Morph	L–Leu	L–Leu	L–Leu	$(OH)_2$	0.14
MG-282	Cbz	—	L–His	L–Leu	$(OH)_2$	25.0
MG-283	Ac	L–Leu	L–Leu	L–Leu	$(OH)_2$	0.46
MG-284		—	—	L–Leu	$(OH)_2$	1.200
MG-285	Morph	—	L–Trp	L–Leu	$(OH)_2$	3.0
MG-286	Morph	—	L–Nal	L–Leu	diethanol-amine	0.15
MG-287	Ac	—	L–Nal	L–Leu	$(OH)_2$	0.13
MG-288	Morph	—	L–Nal	D–Leu	$(OH)_2$	72.5
MG-289	Ms	—	L-(3-Pal)	L–Leu	$(OH)_2$	6.3
MG-290	Ac	—	L-(3-Pal)	L–Leu	$(OH)_2$	5.4
MG-291	Ms	—	L–Nal	L–Leu	diethanol-amine	0.28
MG-292	Morph	—		L–Leu	$(OH)_2$	6.0
MG-293	Morph	—	D–Nal	D–Leu	$(OH)_2$	2,300
MG-294	H	—	L-(3-Pal)	L–Leu	$(OH)_2$	152
MG-295	Ms	—	L–Trp	L–Leu	$(OH)_2$	5.8
MG-296	(8-Quin)-SO_2	—	L–Nal	L–Leu	$(OH)_2$	1.7
MG-297	Ts	—	L–Nal	L–Leu	$(OH)_2$	0.17
MG-298	(2-Quin)-C(O)	—	L–Nal	L–Leu	$(OH)_2$	0.075
MG-299	(2-quinoxalinyl)-C(O)	—	L–Nal	L–Leu	$(OH)_2$	0.14
MG-300	Morph	—	L-(3-Pal)	L–Leu	$(OH)_2$	1.3
MG-301	Ac	—	L–Trp	L–Leu	$(OH)_2$	1.3
MG-302	H	—	L–Nal	L–Leu	$(OH)_2$	7.5
MG-303	H.HCl	—	L–Nal	L–Leu	$(OH)_2$	3.9
MG-304	Ac	L–Leu	L–Nal	L–Leu	$(OH)_2$	0.022
MG-305	Morph	—	D–Nal	L–Leu	$(OH)_2$	189
MG-306	Morph	—	L–Tyr–(O-Benzyl)	L–Leu	$(OH)_2$	0.23
MG-307	Morph	—	L–Tyr	L–Leu	$(OH)_2$	0.51
MG-308	Morph	—	L-(2-Nal)	L–Leu	$(OH)_2$	0.72
MG-309	Morph	—	L–Phe	L–Leu	$(OH)_2$	0.82
MG-310	Ac	—		L–Leu	$(OH)_2$	90
MG-312	Morph	—	L-(2-Pal)	L–Leu	$(OH)_2$	6.3
MG-313	Phenethyl-C(O)	—	—	L–Leu	$(OH)_2$	42

TABLE II-continued

Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
P−AA¹−AA²−AA³−B(Z¹)(Z²)

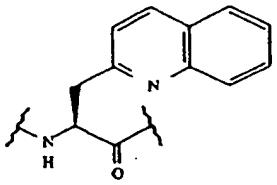
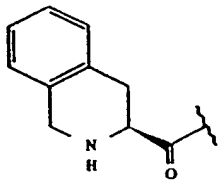
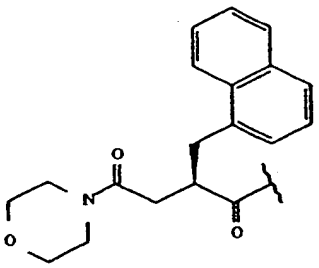
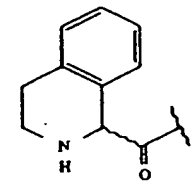
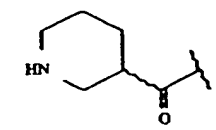
Compound	P*	AA¹	AA²ᵇ	AA³ᶜ	Z¹, Z²	20S K_i (nM)
MG-314	(2-Quin)-C(O)	—	L−Phe	L−Leu	$(OH)_2$	0.19
MG-315	Morph	—		L−Leu	$(OH)_2$	2.2
MG-316	H.HCl	—		L−Leu	$(OH)_2$	22
MG-317	Morph	—	L−Nal	L−Leu	$(OH)(CH_3)$	99
MG-318	Morph	—	L−Nal	L−Leu	$(CH_3)_2$	640
MG-319	H.HCl	—	L−Pro	L−Leu	$(OH)_2$	20
MG-321	Morph	—	L−Nal	L−Phe	$(OH)_2$	0.32
MG-322	Morph	—	L-homoPhe	L−Leu	$(OH)_2$	2.2
MG-323	Ac	—	—	L−Leu	$(OH)_2$	850
MG-324		—	—	L−Leu	H	2.0
MG-325	(2-Quin)-C(O)	—	L-homoPhe	L−Leu	$(OH)_2$	2.8
MG-328	Bz	—	L−Nal	L−Leu	$(OH)_2$	0.088
MG-329	Cyclohexyl-C(O)	—	L−Nal	L−Leu	$(OH)_2$	0.03
MG-332	Cbz(N−Me)	—	L−Nal	L−Leu	$(OH)_2$	0.95
MG-333	H.HCl	—	L−Nal	L−Leu	$(OH)_2$	2.1
MG-334	H.HCl(N−Me)	—	L−Nal	L−Leu	$(OH)_2$	1.1
MG-336	(3-Pyr)−C(O)	—	L−Phe	L−Leu	$(OH)_2$	0.25
MG-337	H.HCl	—		L−Leu	$(OH)_2$	230
MG-338	(2-Quin)-C(O)	—	L-(2-Pal)	L−Leu	$(OH)_2$	1.4
MG-339	H.HCl	—		L−Leu	$(OH)_2$	1,600

TABLE II-continued

Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
P – AA^1 – AA^2 – AA^3 – $B(Z^1)(Z^2)$

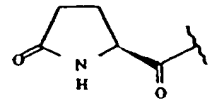
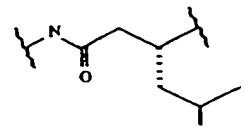
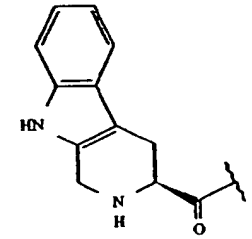
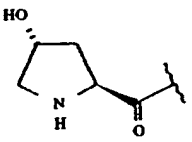
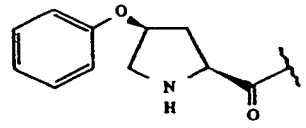
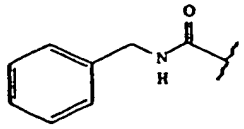
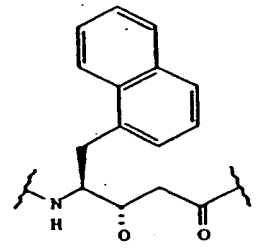
Compound	P*	AA^1	AA^{2b}	AA^{3c}	Z^1, Z^2	20S K_i (uM)
MG-340	H	—		L–Leu	$(OH)_2$	480
MG-341	(2-Pyz)–C(O)	—	L–Phe	L–Leu	$(OH)_2$	0.6
MG-342	Bu	—		—	$(OH)_2$	9,700
MG-343	(2-Pyr)–C(O)	—	L–Phe	L–Leu	$(OH)_2$	0.42
MG-344	Ac	—		L–Leu	$(OH)_2$	51
MG-345	Bz	—	L-(2-Pal)	L–Leu	$(OH)_2$	0.76
MG-346	Cyclohexyl-C(O)	—	L-(2-Pal)	L–Leu	$(OH)_2$	1.1
MG-347	(8-Quin)-SO_2	—	L-(2-Pal)	L–Leu	$(OH)_2$	29
MG-348	H.HCl	—		L–Leu	$(OH)_2$	21
MG-349	H.HCl	—		L–Leu	$(OH)_2$	18
MG-350		—	L–Phe	L–Leu	$(OH)_2$	0.14
MG-351	H.HCl	—	L-(2-Pal)	L–Leu	$(OH)_2$	32
MG-352	Phenylethyl-C(O)	—	L–Phe	L–Leu	$(OH)_2$	0.15
MG-353	Bz	—	L–Phe	L–Leu	$(OH)_2$	0.15
MG-354	(8-Quin)-SO_2	—		L–Leu	$(OH)_2$	28

TABLE II-continued

Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
$P-AA^1-AA^2-AA^3-B(Z^1)(Z^2)$

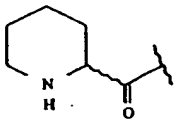
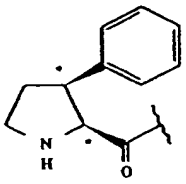
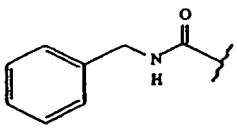
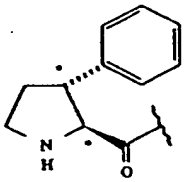
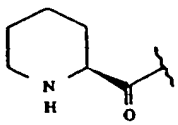
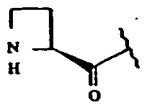
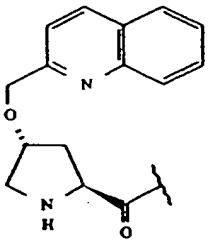
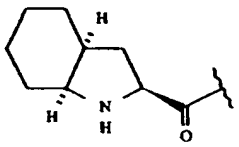
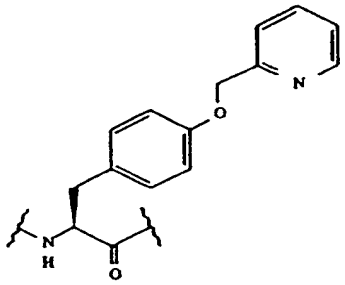
Compound	P[a]	AA^1	AA^{2b}	AA^{3c} Z^1, Z^2	20S K_i (nM)
MG-356	Cbz	—	L-Phe	L-Leu $(OH)_2$	0.13
MG-357	H.HCl	—		L-Leu $(OH)_2$	23
MG-358	(3-Furanyl)-C(O)	—	L-Phe	L-Leu $(OH)_2$	0.17
MG-359	H.HCl	—		L-Leu $(OH)_2$	5.5
MG-361	(3-Pyrrolyl)-C(O)	—	L-Phe	L-Leu $(OH)_2$	0.14
MG-362		—	—	L-Leu $(OH)_2$	6,400
MG-363	H.HCl	—		L-Leu $(OH)_2$	3.45
MG-364	Phenethyl-C(O)	—	—	L-Leu $(OH)_2$	1,500
MG-366	H.HCl	—		L-Leu $(OH)_2$	45.2
MG-368	(2-Pyz)-C(O)	—	L-(2-Pal)	L-Leu $(OH)_2$	5.6
MG-369	H.HCl	—		L-Leu $(OH)_2$	24.2
MG-380	(8-Quin)SO_2	—	L-Phe	L-Leu $(OH)_2$	4.4
MG-382	(2-Pyz)-C(O)	—	L-(4-F)-Phe	L-Leu $(OH)_2$	0.95
MG-383	(2-Pyr)-C(O)	—	L-(4-F)-Phe	L-Leu $(OH)_2$	0.84

TABLE II-continued

Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
P—AA¹—AA²—AA³—B(Z¹)(Z²)

Compound	P[a]	AA¹	AA²[b]	AA³	Z¹, Z²	20S K_i (nM)
MG-385	H.HCl	—		L—Leu	$(OH)_2$	23
MG-386	H.HCl	—		L—Leu	$(OH)_2$	92
MG-387	Morph	—		L—Leu	$(OH)_2$	0.2

[a]Cbz = carbobenzyloxy; MS = methylsulfonyl; Morph = 4-morpholinecarbonyl; (8-Quin)-SO_2 = 8-quinolinesulfonyl; (2-Quin)-C(O) = 2-quinolinecarbonyl; Bz = benzoyl; (2-Pyr)—C(O) = 2-pyridinecarbonyl; (3-Pyr)—C(O) = 3-pyridinecarbonyl; (2-Pyz)—C(O) = 2-pyrazinecarbonyl.
[b]Nal = β-(1-naphthyl)alanine; (2-Nal) = β-(2-naphthyl)alanine; (2-Pal) = β-(2-pyridyl)alanine; (3-Pal) = β-(3-pyridyl)alanine; homoPhe = homophenylalanine; (4-F)-Phe = (4-fluorophenyl)alanine.
[c]B(Z¹)(Z²) takes the place of the carboxyl group of AA³.

In Table III, P, AA¹, AA², AA³, and X are substituents of the general formula: P - AA¹- AA²- AA³- X

Table III demonstrates that dipeptide boronic acids have lower K_i values than the corresponding dipeptide aldehydes.

TABLE III

Comparison of Dipeptide Boronic Acids to Dipeptide Aldehydes

Cpd.	P	AA¹	AA²	AA³	X	20S K_i(nM)
MG-105	Z	—	L—Leu	L—Leu	CHO	15,000
MG-274	Z	—	L—Leu	L—Leu	$B(OH)_2$	3.0

In Table IV, P, AA¹, AA², AA³, and X are substituents of the general formula: P - AA¹- AA²- AA³- X.

Table IV demonstrates the markedly superior selectivity for the 20S proteasome over other proteases, e.g. Cathepsin B, exhibited by the boronic esters/acids as compared to the peptide aldehydes.

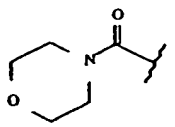
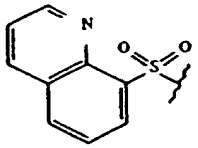
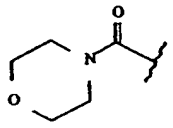
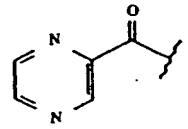
TABLE IV

Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
P—AA¹—AA²—AA³—X

Compound	P	AA¹	AA²	AA³	X	20S K_i (nM)	Cathepsin B K_i (nM)
MG-154	Ac	L—Leu	L—Leu	L—Leu	CHO	66.0	5.0
MG-191	Cbz	L—Trp	L—Leu	L—Leu	CHO	0.38	0.54

TABLE IV-continued

Inhibition of the 20S Proteasome by Boronic Ester and Acid Compounds
P−AA¹−AA²−AA³−X

Compound	P	AA¹	AA²	AA³	X	20S K_i (nM)	Cathepsin B K_i (nM)
MG-262	Cbz	L−Leu	L−Leu	L−Leu	$B(OH)_2$	0.035	6,100
MG-273		—	L−Nal	L−Leu	$B(OH)_2$	0.18	200,000
MG-296		—	L−Nal	L−Leu	$B(OH)_2$	1.7	4,000
MG-309		—	L−Phe	L−Leu	$B(OH)_2$	0.82	132,000
MG-341		—	L−Phe	L−Leu	$B(OH)_2$	0.6	160,000

The selectivity of boronic acid inhibitors of the proteasome is further demonstrated in Table V.

TABLE V

Selectivity of Boronic Ester and Acid Inhibitors of the 20S Proteasome

Compound	20S K_i (nM)	Human Leukocyte Elastase K_i (nM)	Cathepsin G K_i (nM)	Human Pancreatic Chymotrypsin K_i (nM)
MG-262	0.03	15	55	7
MG-267	0.1	150	33,000	2,300
MG-296	1.7	36	9,200	75
MG-309	0.82	7,000	4,800	465
MG-341	0.6	2,300	628	322

EXAMPLE 19

Inhibition of Protein Degradation in C2C12 Cells

C2C12 cells (a mouse myoblast line) were labelled for 48 hrs with ^{35}S-methionine. The cells were then washed and preincubated for 2 hrs in the same media supplemented with 2 mM unlabelled methionine. The media was removed and replaced with a fresh aliquot of the preincubation media containing 50% serum, and a concentration of the compound to be tested. The media was then removed and made up to 10% TCA and centrifuged. The TCA soluble radioactivity was counted. Inhibition of proteolysis was calculated as the percent decrease in TCA soluble radioactivity. From this data, an EC_{50} for each compound was calculated.

Data for compounds of formula (1) or (2) are presented in Table VI.

TABLE VI

Inhibition of Protein Degradation in C2C12 Cells by Boronic Ester and Acid Compounds
P−AA¹−AA²−AA³−B(Z¹)(Z²)

Compound	P*	AA¹	AA²*	AA³*	Z¹, Z²	IC_{50} (nM)
MG-262	Cbz	L−Leu	L−Leu	L−Leu	$(OH)_2$	280
MG-270	Cbz	—	L−Nal	L−Leu	$(OH)_2$	730
MG-272	Cbz	—	D-(2-Nal)	L−Leu	$(OH)_2$	6,000
MG-273	Morph	—	L−Nal	L−Leu	$(OH)_2$	140
MG-274	Cbz	—	L−Leu	L−Leu	$(OH)_2$	340
MG-278	Morph	L−Leu	L−Leu	L−Leu	$(OH)_2$	7,500
MG-282	Cbz	—	L−His	L−Leu	$(OH)_2$	64,000
MG-283	Ac	L−Leu	L−Leu	L−Leu	$(OH)_2$	3,000
MG-285	Morph	—	L−Trp	L−Leu	$(OH)_2$	2,400

TABLE VI-continued

Inhibition of Protein Degradation in C2C12 Cells by Boronic Ester and Acid Compounds
P—AA^1—AA^2—AA^3—$B(Z^1)(Z^2)$

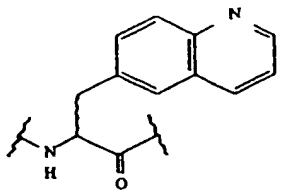
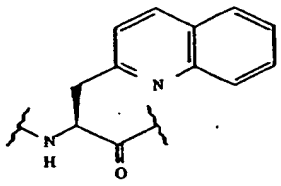
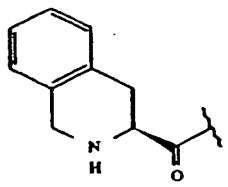
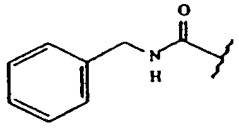
Compound	P*	AA^1	AA^{2b}	AA^{3c} Z^1, Z^2	IC_{50} (nM)
MG-286	Morph	—	L—Nal	L—Leu diethanolamine	95
MG-287	Ac	—	L—Nal	L—Leu $(OH)_2$	106
MG-289	Ms	—	L-(3-Pal)	L—Leu $(OH)_2$	10,830
MG-290	Ac	—	L-(3-Pal)	L—Leu $(OH)_2$	10,240
MG-292	Morph	—		L—Leu $(OH)_2$	11,320
MG-296	(8-Quin)-SO_2	—	L—Nal	L—Leu $(OH)_2$	738
MG-298	(2-Quin)-C(O)	—	L—Nal	L—Leu $(OH)_2$	230
MG-299	(2-Quinoxalinyl)-C(O)	—	L—Nal	L—Leu $(OH)_2$	280
MG-301	Ac	—	L—Trp	L—Leu $(OH)_2$	1,300
MG-302	H	—	L—Nal	L—Leu $(OH)_2$	270
MG-303	H.HCl	—	L—Nal	L—Leu $(OH)_2$	340
MG-304	Ac	L—Leu	L—Nal	L—Leu $(OH)_2$	240
MG-306	Morph	—	L—Tyr—(O—Bn)	L—Leu $(OH)_2$	130
MG-307	Morph	—	L—Tyr	L—Leu $(OH)_2$	4,800
MG-308	Morph	—	L-(2-Nal)	L—Leu $(OH)_2$	96
MG-309	Morph	—	L—Phe	L—Leu $(OH)_2$	210
MG-312	Morph	—	L-(2-Pal)	L—Leu $(OH)_2$	1,100
MG-313	Phenethyl-C(O)	—	—	L—Leu $(OH)_2$	3,500
MG-314	(2-Quin)-C(O)	—	L—Phe	L—Leu $(OH)_2$	130
MG-315	Morph	—		L—Leu $(OH)_2$	340
MG-316	H.HCl	—		L—Leu $(OH)_2$	21,000
MG-319	H.HCl	—	L—Pro	L—Leu $(OH)_2$	14,000
MG-321	Morph	—	L—Nal	L—Phe $(OH)_2$	2,400
MG-322	Morph	—	L-homoPhe	L—Leu $(OH)_2$	380
MG-325	(2-Quin)-C(O)	—	L-homoPhe	L—Leu $(OH)_2$	1,100
MG-328	Bz	—	L—Nal	L—Leu $(OH)_2$	69
MG-329	Cyclohexyl-C(O)	—	L—Nal	L—Leu $(OH)_2$	48
MG-332	Cbz(N—Me)	—	L—Nal	L—Leu $(OH)_2$	950
MG-333	H.HCl	—	L—Nal	L—Leu $(OH)_2$	220
MG-334	H.HCl(N—Me)	—	L—Nal	L—Leu $(OH)_2$	320
MG-336	(3-Pyr)—C(O)	—	L—Phe	L—Leu $(OH)_2$	100
MG-341	(2-Pyz)—C(O)	—	L—Phe	L—Leu $(OH)_2$	69
MG-343	(2-Pyr)—C(O)	—	L—Phe	L—Leu $(OH)_2$	57
MG-345	Bz	—	L-(2-Pal)	L—Leu $(OH)_2$	120
MG-346	Cyclohexyl-C(O)	—	L-(2-Pal)	L—Leu $(OH)_2$	150
MG-347	(8-Quin)-SO_2	—	L-(2-Pal)	L—Leu $(OH)_2$	13,000

TABLE VI-continued

Inhibition of Protein Degradation in C2C12 Cells by Boronic Ester and Acid Compounds
P—AA^1—AA^2—AA^3—$B(Z^1)(Z^2)$

Compound	P[a]	AA^1	AA^{2b}	AA^3	Z^1, Z^2	IC_{50} (nM)
MG-350		—	L—Phe	L—Leu	$(OH)_2$	160
MG-351	H.HCl	—	L-(2-Pal)	L—Leu	$(OH)_2$	8,100

[a]Cbz = carbobenzyloxy; Morph = 4-morpholinecarbonyl; (8-Quin)SO_2 = 8-quinolinesulfonyl; (2-Quin)C(O) = 2-quinolinecarbonyl; Bz = benzoyl; (2-Pyr)—C(O) = 2-pyridinecarbonyl; (3-Pyr)—C(O) = 3-pyridinecarbonyl; (2-Pyz)—C(O) = 2-pyrazinecarbonyl.
[b]Nal = β-(1-naphthyl)alanine; (2-Nal) = β-(2-naphthyl)alanine; (2-Pal) = β-(2-pyridyl)alanine; (3-Pal) = b-(3-pyridyl)alanine; homoPhe = homophenylalanine.
[c]$B(Z^1)(Z^2)$ takes the place of the carboxyl group of AA^3.

EXAMPLE 20

MG-273 Inhibits Corticosterone-Induced Cachexia in Rats

Rats were stabilized on a diet free from 3-methylhistidine and then placed in metabolic cages for collection of 24-hour urine samples. After two days of urine collections to determine basal 3-methylhistidine output, the rats were treated with daily subcutaneous injections of corticosterone (100 mg/kg). Starting on the second day of corticosterone treatment, some of the rats were also treated with MG-273, administered via a subcutaneous osmotic pump at a dose rate of approximately 120 μg/kg body weight/day. Control rats received vehicle only (25% DMSO/75% PEG (200)), administered in a similar fashion. FIG. 1 shows that treatment with MG-273 reduced the urinary output of 3-methylhistidine, which was induced in response to corticosterone treatment.

EXAMPLE 21

MG-273 Inhibits the Activation of NF-κB

This assay was performed as previously described (Palombella, et al. *Cell*, 78.773–785 (1994)). MG63 osteosarcoma cells were stimulated by treatment with TNF-α for the designated times. Whole cell extracts were prepared and analyzed by electrophoretic mobility shift assay using the PRDII probe from the human IFN-β gene promoter. FIG. 2 shows that NF-κB binding activity was inhibited by pretreatment for 1 hour with MG 273. An aldehyde inhibitor of the proteasome, MG-132 (Cbz-L-Leu-L-Leu-L-Leu-H), also inhibited NF-κB binding activity, whereas MG- 102 (Ac-L-Leu-L-Leu-L-Met-H), which is inactive against the 20S proteasome, did not inhibit NF-κB binding activity.

EXAMPLE 22

MG-273 Inhibits Expression of Cell Adhesion Molecules on HUVE Cells

HUVECs in microtiter plates were exposed to the indicated concentrations of inhibitor for 1 hour, prior to the addition of 100 U/mL TNF-α. Cell surface binding assays were performed at 4° C., using saturating concentrations of monoclonal antibodies specific for the cell adhesion molecules (Becton Dickenson) and fluorescent-conjugated $F(ab')_2$ goat anti-murine IgG (Caltag Labs, San Francisco, Calif.). Fluorescent immunoassays for E-selectin and I-CAM were performed at 4 hours, those for V-CAM at 16 hours. FIG. 3 shows that cell-surface expression I-CAM, V-CAM, and E-selectin on TNF-α stimulated HUVECs is significantly inhibited by MG-273 at concentrations of 0.5 μM or above.

EXAMPLE 23

Boronic Acid Compounds Block the DTH Response in Mice

Naive mice were sensitized by the application of 20 μL of a 0.5% (v/v) solution of 2,4-dinitrofluorobenzene in 4:1 acetone/olive oil to both of the rear limb footpads. This procedure is performed on two consecutive days, which are referred to as days 0 and 1.

The efferent phase of the contact sensitivity response was elicited on day 5 by the application of 10 μL of a 0.2% (v/v) solution of 2,4-dinitrofluorobenzene in 4:1 acetone/olive oil to both sides of the left ear. The contralateral control ear was treated on both sides with 10 μL of vehicle only. The mice were lightly anaesthetized for this procedure by the intraperitoneal (i.p.) injection of a mixture of ketamine (80 mg/kg, Henry Schein) and xylazine (16 mg/kg, Henry Schein).

Test compounds were administered orally as a suspension in 0.5% methylcellulose (4000 centipoises Fisher Scientific) 30 minutes prior to the application of the challenge dose of 2,4-dinitrofluorobenzene to the ears. The dose was delivered in a final volume of 0.5 mL using a 24 gauge 1 inch malleable feeding needle with a 1.25 mm ball tip (Roboz Surgical).

Approximately 18 hours after the challenge, ear swelling was determined by measuring both the control and the experimental ear using a Mitutoyo Digital micrometer. The absolute difference in thickness of the experimental (left) ears vs. the control (right) ears was determined for each treatment group. Efficacy was determined by comparing this difference in thickness to the difference calculated for the vehicle control group. Test results are provided in Table VII.

TABLE VII

Inhibition of the DTH Response in Mice

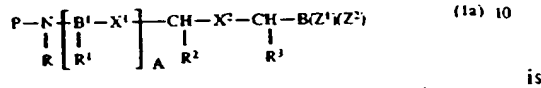
Compound	Dose (mg/kg)	% Inhibition
MG-296	50	60
MG-309	3	40
MG-341	3	90

All publications and U.S. patent applications mentioned hereinabove are hereby incorporated in their entirety by reference.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

What is claimed is:

1. A compound having the formula:



(Ia)

or a pharmaceutically acceptable salt thereof; wherein

P is R^7—C(O)— or R^7—SO_2—, where R^7 is pyrazinyl;

X^2 is —C(O)—NH—;

R is hydrogen or alkyl;

R^2 and R^3 are independently hydrogen, alkyl, cycloalkyl, aryl, or —CH_2—R^5;

R^5, in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, or —W—R^6, where W is a chalcogen and R^6 is alkyl;

where the ring portion of any of said aryl, aralkyl, or alkaryl in R^2, R^3 and R^5 can be optionally substituted by one or two substituents independently selected from the group consisting of C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{1-6} alkyl(C_{3-8})cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, cyano, amino, C_{1-6} alkylamino, di(C_{1-6})alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6})alkoxy, trifluoromethyl, halogen, C_{1-6} alkoxy, C_{6-10} aryl, C_{6-10} aryl(C_{1-6})alkyl, C_{6-10} aryl(C_{1-6})alkoxy, hydroxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{6-10} arylthio, C_{6-10} arylsulfinyl, C_{6-10} arylsulfonyl, C_{6-10} aryl, C_{1-6} alkyl(C_{6-10}) aryl, and halo(C_{6-10})aryl;

Z^1 and Z^2 are independently one of hydroxy, alkoxy, or aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising carbon atoms, and optionally, a heteroatom or heteroatoms which can be N, S, or O; and

A is zero.

2. The compound of claim 1, wherein:

A is zero;

X is —C(O)—NH—;

R is hydrogen or C_{1-8} alkyl; and

R_3 is C_{1-6} alkyl.

3. The compound of claim 2, wherein R_3 is C_4alkyl.

4. The compound of claim 1, wherein P is one of 2-pyrazinecarbonyl, or 2-pyrazinesulfonyl.

5. The compound of claim 1, wherein R is hydrogen or C_{1-8} alkyl.

6. The compound of claim 1, wherein:

R^2 and R^3 are each independently one of hydrogen, C_{1-8} alkyl, C_{3-10} cycloalkyl, or C_{6-10} aryl, or —CH_2—R^5;

R^5, in each instance, is one of C_{6-10}aryl, C_{6-10} ar(C_{1-6}) alkyl, C_{1-6}alk(C_{6-10})aryl, C_{3-10} cycloalkyl, C_{1-8} alkoxy, or C_{1-8}alkylthio;

where the ring portion of any of said aryl, aralkyl, or alkaryl groups of R^2, R^3 and R^5 can be optionally substituted by one or two substituents independently selected from the group consisting of C_{1-6}alkyl, C_{3-8}cycloalkyl, C_{1-6}alkyl(C_{3-8})cycloalkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, cyano, amino, C_{1-6}alkylamino, di(C_{1-6}) alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6})alkoxy, trifluoromethyl, halogen, C_{1-6}alkoxy, C_{6-10}aryl, C_{6-10}aryl(C_{1-6})alkyl, C_{6-10}aryl (C_{1-6})alkoxy, hydroxy, C_{1-6}alkylthio, C_{1-6}alkylsulfinyl, C_{1-6}alkylsulfonyl, C_{6-10}arylthio, C_{6-10}arylsulfinyl, C_{6-10}arylsulfonyl, C_{6-10}aryl, C_{1-6}alkyl(C_{6-10})aryl, and halo(C_{6-10})aryl.

7. The compound of claim 1, wherein R_3 is C_{1-12}alkyl.

8. The compound of claim 1, wherein R_3 is C_{1-6}alkyl.

9. The compound of claim 1, wherein R_3 is C_4alkyl.

10. The compound of claim 1, wherein R^3 is isobutyl.

11. The compound of claim 1, wherein R^2 is one of isobutyl, 1-naphthylmethyl, 2-naphthylmethyl, benzyl, 4-fluorobenzyl, 4-hydroxybenzyl, 4-(benzyloxy)benzyl, benzylnaphthylmethyl or phenethyl.

12. The compound of claim 1, wherein Z^1 and Z^2 are independently one of hydroxy, C_{1-6}alkoxy, or C_{6-10}aryloxy.

13. The compound of claim 12, wherein Z^1 and Z^2 are both hydroxy.

14. The compound of claim 1, wherein together Z^1 and Z^2 form a moiety derived from a dihydroxy compound selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol or diethanolamine.

15. The compound of claim 1, wherein:

P is one of quinolinecarbonyl, pyridinecarbonyl, quinolinesulfonyl, quinoxalinecarbonyl, quinoxalinesulfonyl, pyrazinecarbonyl, pyrazinesulfonyl, furancarbonyl, furansulfonyl or N-morpholinylcarbonyl;

A is zero;

X^2 is —C(O)—NH—;

R is hydrogen or C_{1-8} alkyl;

R^2 and R^3 are each independently one of hydrogen, C_{1-8}alkyl, C_{3-10}cycloalkyl, C_{6-10}aryl, C_{6-10}ar(C_{1-6})alkyl, pyridylmethyl, or quinolinylmethyl; and

Z^1 and Z^2 are both hydroxy, C_{1-6}alkoxy, or C_{6-10}aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol or diethanolamine.

16. The compound of claim 1, wherein:

P is one of 2-pyrazinecarbonyl, or 2-pyrazinesulfonyl;

A is zero;

X^2 is —C(O)—NH—;

R is hydrogen or C_{1-8} alkyl;

R^3 is isobutyl;

R^2 is one of isobutyl, 1-naphthylmethyl, 2-naphthylmethyl, benzyl, 4-fluorobenzyl, 4-hydroxybenzyl, 4-(benzyloxy)benzyl, benzylnaphthylmethyl or phenethyl; and

Z^1 and Z^2 are independently one of hydroxy, C_{1-6}alkoxy, C_{6-10}aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol and diethanolamine.

17. The compound of claim 1, wherein said compound is one of:

N-(2-pyrazine)carbonyl-L-phenylalanine-L-leucine boronic acid.

N-(2-quinoline)sulfonyl-L-homophenylalanine-L-leucine boronic acid.

N-(3-pyridine)carbonyl-L-phenylalanine-L-leucine boronic acid.

N-(4-morpholine)carbonyl-L-phenylalanine-L-leucine boronic acid.

N-(4-morpholine)carbonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid.

N-(8-quinoline)sulfonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid.

N-(4-morpholine)carbonyl-(O-benzyl)-L-tyrosine-L-leucine boronic acid.

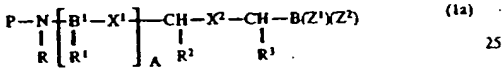
N-(4-morpholine)carbonyl-L-tyrosine-L-leucine boronic acid.

N-(4-morpholine)carbonyl-[O-(2-pyridylmethyl)]-L-tyrosine-L-leucine boronic acid;

or isosteres, pharmaceutically acceptable salts or boronate esters thereof.

18. The compound N-(2-pyrazine)carbonyl-L-phenylalanine-L-leucine boronic acid, or a pharmaceutically acceptable salt or boronate ester thereof.

19. A compound having the formula:



(1a)

or a pharmaceutically acceptable salt thereof; wherein

P is R^7—C(O)— and R^7 is pyrazinyl;

X^2 is —C(O)—NH—;

R is hydrogen or alkyl;

R^2 and R^3 are independently hydrogen, alkyl, cycloalkyl, aryl, or —CH_2—R^5;

R^5, in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, or —W—R^6, where W is a chalcogen and R^6 is alkyl;

where the ring portion of any of said aryl, aralkyl, or alkaryl in R^2, R^3 and R^5 can be optionally substituted by one or two substituents independently selected from the group consisting of C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{1-6} alkyl(C_{3-8})cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, cyano, amino, C_{1-6} alkylamino, di(C_{1-6})alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6})alkoxy, trifluoromethyl, halogen, C_{1-6} alkoxy, C_{6-10} aryl, C_{6-10} aryl(C_{1-6})alkyl, C_{6-10} aryl(C_{1-6})alkoxy, hydroxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{6-10} arylthio, C_{6-10} arylsulfinyl, C_{6-10} arylsulfonyl, C_{6-10} aryl, C_{1-6}alkyl (C_{6-10})aryl, and halo (C_{6-10})aryl;

Z^1 and Z^2 are independently one of hydroxy, alkoxy, or aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising carbon atoms, and optionally, a heteroatom or heteroatoms which can be N, S, or O; and

A is zero.

20. A pharmaceutical composition, comprising a compound of claim **1**, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

21. A pharmaceutical composition, comprising a compound of claim **19**, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

22. The pharmaceutical composition of claim **20** or **21**, wherein said compound is present in an amount effective to inhibit the proteasome function in a mammal.

* * * * *

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
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M12DM

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HALE AND DORR LLP
60 STATE STREET
BOSTON MA 02109

HALE AND DORR LLP
JAN 23 2002
INTELLECTUAL PROPERTY DEPARTMENT

MAINTENANCE FEE STATEMENT

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 11, "STAT" below.

If a maintenance fee payment is defective, the reason is indicated by code in column 11, "STAT" below. TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(h).

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

ITEM NBR	PATENT NUMBER	FEE CDE	FEE AMT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT
1	5,780,454	283	440		08/549,318	07/14/98	10/27/95	04	YES	PAID

HALE & DORR DOCKETING
RE: 103576.135 US3
Action Date: 1.14.06
Action to be Taken: "OA"
Docketed by: AG On: 1.23.02

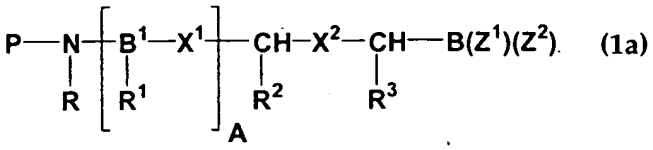
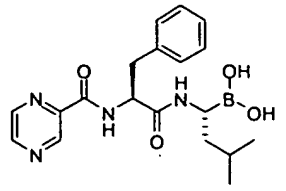
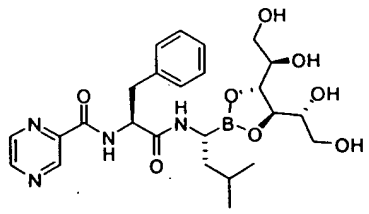
ITM NBR	ATTY DKT NUMBER
1	1448.0120002

DIRECT THE RESPONSE TOGETHER WITH ANY QUESTIONS ABOUT THIS NOTICE TO: COMMISIONER OF PATENTS AND TRADEMARKS, BOX M. FEE, WASHINGTON, D.C. 20231

E

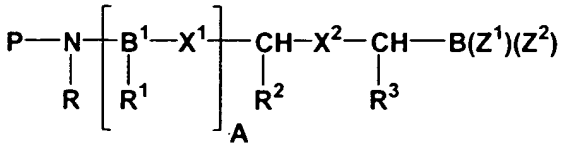
Exhibit E

Claim Chart

1. A compound having the formula:  (1a) or a pharmaceutically acceptable salt thereof; wherein P is R^7–C(O)– or R^7–SO_2–, where R^7 is pyrazinyl; X^2 is –C(O)–NH–; R is hydrogen or alkyl; R^2 and R^3 are independently hydrogen, alkyl, cycloalkyl, aryl, or –CH_2–R^5; R^5, in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, or –W–R^6, where W is a chalcogen and R^6 is alkyl; where the ring portion of any of said aryl, aralkyl, or alkaryl in R^2, R^3 and R^5 can be optionally substituted by one or two substituents independently selected from the group consisting of C_{1-6} alkyl, C_{3-8} cycloalkyl, C_{1-6}alkyl(C_{3-8})cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, cyano, amino, C_{1-6} alkylamino, di(C_{1-6})alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6})alkoxy, trifluoromethyl, halogen, C_{1-6} alkoxy, C_{6-10} aryl, C_{6-10} aryl(C_{1-6})alkyl, C_{6-10} aryl(C_{1-6})alkoxy, hydroxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{6-10} arylthio, C_{6-10} arylsulfinyl, C_{6-10} arylsulfonyl, C_{6-10} aryl, C_{1-6} alkyl(C_{6-10})aryl, and halo(C_{6-10})aryl; Z^1 and Z^2 are independently one of hydroxy, alkoxy, or aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising carbon atoms and, optionally, a heteroatom or heteroatoms which can be N, S, or O; and A is zero.	**Bortezomib** (active ingredient)  P is R^7-C(O)-, where R^7 is pyrazinyl; X^2 is –C(O)-NH-; R is hydrogen; R^2 is –CH_2-R^5, where R^5 is phenyl (*i.e.*, aryl); R^3 is isobutyl (*i.e.*, alkyl); Z^1 and Z^2 are both hydroxy; A is zero. **VELCADE™ (bortezomib) for Injection** (formulated drug product)  P is R^7-C(O)-, where R^7 is pyrazinyl; X^2 is –C(O)-NH-; R is hydrogen; R^2 is –CH_2-R^5, where R^5 is phenyl (*i.e.*, aryl); R^3 is isobutyl (*i.e.*, alkyl); together Z^1 and Z^2 form a moiety derived from mannitol (*i.e.*, a dihydroxy compound) having six hydroxy groups (*i.e.*, at least two hydroxy groups) separated by two connecting atoms in a chain,

	said chain comprising carbon atoms; A is zero.
2. The compound of claim 1, wherein: A is zero; X is –C(O)–NH–; R is hydrogen or C_{1-8} alkyl; and R^3 is C_{1-6} alkyl.	
3. The compound of claim 2, wherein R_3 is C_4 alkyl.	
4. The compound of claim 1, wherein P is one of 2-pyrazinecarbonyl, or 2-pyrazinesulfonyl.	
5. The compound of claim 1, wherein R is hydrogen or C_{1-8} alkyl.	
6. The compound of claim 1, wherein: R^2 and R^3 are each independently one of hydrogen, C_{1-8} alkyl, C_{3-10} cycloalkyl, or C_{6-10} aryl, or $-CH_2-R^5$; R^5, in each instance, is one of C_{6-10} aryl, C_{6-10} ar(C_{1-6}) alkyl, C_{1-6}alk (C_{6-10})aryl, C_{3-10} cycloalkyl, C_{1-8} alkoxy, or C_{1-8}alkylthio; where the ring portion of any of said aryl, aralkyl, or alkaryl groups of R^2, R^3 and R^5 can be optionally substituted by one or two substituents independently selected from the group consisting of C_{1-6}alkyl, C_{3-8} cycloalkyl, C_{1-6}alkyl(C_{3-8})cycloalkyl, C_{2-8}alkenyl, C_{2-8}alkynyl, cyano, amino, C_{1-6}alkylamino, di(C_{1-6})alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6})alkoxy, trifluoromethyl, halogen, C_{1-6}alkoxy, C_{6-10}aryl, C_{6-10}aryl(C_{1-6})alkyl, C_{6-10}aryl(C_{1-6})alkoxy, hydroxy, C_{1-6}alkylthio, C_{1-6}alkylsulfinyl, C_{1-6}alkylsulfonyl, C_{6-10}arylthio, C_{6-10}arylsulfinyl, C_{6-10}arylsulfonyl, C_{6-10}aryl, C_{1-6}alkyl(C_{6-10})aryl, and halo(C_{6-10})aryl.	
7. The compound of claim 1, wherein R_3 is C_{1-12}alkyl.	
8. The compound of claim 1, wherein R_3 is C_{1-6}alkyl.	
9. The compound of claim 1, wherein R_3 is C_4alkyl.	
10. The compound of claim 1, wherein R^3 is isobutyl.	

11. The compound of claim 1, wherein R^2 is one of isobutyl, 1-naphthylmethyl, 2-naphthylmethyl, benzyl, 4-fluorobenzyl, 4-hydroxybenzyl, 4-(benzyloxy)benzyl, benzylnaphthylmethyl or phenethyl.	
12. The compound of claim 1, wherein Z^1 and Z^2 are independently one of hydroxy, C_{1-6}alkoxy, or C_{6-10}aryloxy.	
13. The compound of claim 12, wherein Z^1 and Z^2 are both hydroxy.	
15. The compound of claim 1, wherein: P is one of quinolinecarbonyl, pyridinecarbonyl, quinolinesulfonyl, quinoxalinecarbonyl, quinoxalinesulfonyl, pyrazinecarbonyl, pyrazinesulfonyl, furancarbonyl, furansulfonyl or N-morpholinylcarbonyl; A is zero; X^2 is –C(O)-NH-; R is hydrogen or C_{1-8} alkyl; R^2 and R^3 are each independently one of hydrogen, C_{1-8}alkyl, C_{3-10}cycloalkyl, C_{6-10}aryl, C_{6-10}ar(C_{1-6})alkyl, pyridylmethyl, or quinolinylmethyl; and Z^1 and Z^2 are both hydroxy, C_{1-6}alkoxy, or C_{6-10}aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol or diethanolamine.	
16. The compound of claim 1, wherein: P is one of 2-pyrazinecarbonyl, or 2-pyrazinesulfonyl; A is zero; X^2 is –C(O)-NH-; R is hydrogen or C_{1-8} alkyl; R^3 is isobutyl; R^2 is one of isobutyl, 1-naphthylmethyl, 2-naphthylmethyl, benzyl, 4-fluorobenzyl, 4-hydroxybenzyl, 4-(benzyloxy)benzyl, benzylnaphthylmethyl or phenethyl; and Z^1 and Z^2 are independently one of hydroxy, C_{1-6}alkoxy, C_{6-10}aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound	

selected from the group consisting of pinacol, perfluoropinacol, pinanediol, ethylene glycol, diethylene glycol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, glycerol and diethanolamine.	
17. The compound of claim 1, wherein said proteasome inhibitor is one of: N-(2-pyrazine)carbonyl-L-phenylalanine-L-leucine boronic acid, N-(2-quinoline)sulfonyl-L-homophenylalanine-L-leucine boronic acid; N-(3-pyridine)carbonyl-L-phenylalanine-L-leucine boronic acid, N-(4-morpholine)carbonyl-L-phenylalanine-L-leucine boronic acid, N-(4-morpholine)carbonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid. N-(8-quinoline)sulfonyl-β-(1-naphthyl)-L-alanine-L-leucine boronic acid, N-(4-morpholine)carbonyl-(O-benzyl)-L-tyrosine-L-leucine boronic acid, N-(4-morpholine)carbonyl-L-tyrosine-L-leucine boronic acid, N-(4-morpholine)carbonyl-[O-(2-pyridylmethyl)]-L-tyrosine-L-leucine boronic acid; or an isostere, pharmaceutically acceptable salt or boronate ester thereof.	
18. The compound N-(2-pyrazine)carbonyl-L-phenylalanine-L-leucine boronic acid, or a pharmaceutically acceptable salt or boronate ester thereof.	
19. A compound having the formula:  (1a) or a pharmaceutically acceptable salt thereof; wherein P is R^7-C(O)- and R^7 is pyrazinyl; X^2 is –C(O)-NH-; R is hydrogen or alkyl; R^2 and R^3 are independently hydrogen, alkyl,	

cycloalkyl, aryl, or $-CH_2-R^5$; R^5, in each instance, is one of aryl, aralkyl, alkaryl, cycloalkyl, or $-W-R^6$, where W is a chalcogen and R^6 is alkyl; where the ring portion of any of said aryl, aralkyl, or alkaryl in R^2, R^3 and R^5 can be optionally substituted by one or two substituents independently selected from the group consisting of C_{1-6}alkyl, C_{3-8}cycloalkyl, C_{1-6}alkyl(C_{3-8})cycloalkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, cyano, amino, C_{1-6} alkylamino, di(C_{1-6})alkylamino, benzylamino, dibenzylamino, nitro, carboxy, carbo(C_{1-6})alkoxy, trifluoromethyl, halogen, C_{1-6} alkoxy, C_{6-10}aryl, C_{6-10} aryl(C_{1-6})alkyl, C_{6-10} aryl(C_{1-6})alkoxy, hydroxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} alkylsulfonyl, C_{6-10} arylthio, C_{6-10} arylsulfinyl, C_{6-10} arylsulfonyl, C_{6-10}aryl, C_{1-6}alkyl (C_{6-10})aryl, and halo (C_{6-10})aryl; Z^1 and Z^2 are independently one of hydroxy, alkoxy, or aryloxy, or together Z^1 and Z^2 form a moiety derived from a dihydroxy compound having at least two hydroxy groups separated by at least two connecting atoms in a chain or ring, said chain or ring comprising carbon atoms, and optionally, a heteroatom or heteroatoms which can be N, S, or O; and A is zero.	
20. A pharmaceutical composition, comprising a compound of claim **1**, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.	
21. A pharmaceutical composition, comprising a compound of claim **19**, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.	
22. The pharmaceutical composition of claim **20** or **21**, wherein said compound is present in an amount effective to inhibit the proteasome function in a mammal.	

F

PS-341 Submissions/Correspondence Index

Date	Serial No.	Doc Type	To	From	Description	Location
5-20-03		FDA Correspondence	Tanya Lewis	Robert Temple	Approval Letter.	56
5-20-03	276	SAE Report	Sean Bradley	Tanya Lewis	Manufacturer Report Nos. S03-341-278 and S03-341-230.	56
5-19-03		DDMAC Faxed correspondence	Tanya Lewis	Catherine Miller	DDMAC comments on draft materials.	56
5-19-03	275	Sponsor Correspondence	Richard Pazdur	Renu Vaish	Authorize FDA to reference our IND Application and subsequent submissions.	56
5-16-03		DDMAC Correspondence	Tanya Lewis	Catherine Miller	Draft Press Release	56
5-16-03		DDMAC Mailed Correspondence	Tanya Lewis	Catherine Miller	Comments on draft promotional materials.	56
5-16-03		DDMAC Mailed Correspondence	Tanya Lewis	Catherine Miller	Comments on draft video news release.	56

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Date	Serial No.	Doc Type	To	From	Description	Location
5-15-03	273	SAE Report	Sean Bradley	Renu Vaish	1571 for SAE Reports submitted on 5-12-03.	56
5-15-03	274	SAE Reports	Sean Bradley	Tanya Lewis	Manufacturer Report No. S03-341-228.	56
5-13-03		Sponsor Correspondence	Catherine Miller	Tanya Lewis	Comments added to press release. For Cathy's review.	56
5-13-03		Faxed FDA Correspondence	Tanya Lewis	Sean Bradley	Approval Letter	56
5-13-03		Faxed DDMAC Correspondence	Tanya Lewis	Catherine Miller	Comments on draft video news release.	56
5-13-03		Faxed DDMAC Correspondence	Tanya Lewis	Catherine Miller	Comments on draft press release.	56
5-12-03		Faxed DDMAC Correspondence	Tanya Lewis	Catherine Miller	Comments on draft promotional materials.	55

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Date	Serial No.	Doc Type	To	From	Description	Location
5-12-03	273	SAE Report	Sean Bradley	Renu Vaish	Manufacturer Report Nos. S03-341-230 & S03-341-262.	55
5-7-03		FDA Correspondence	Tanya Lewis	Sean Bradley	FDA - Revised version of labeling.	55
5-7-03	272	SAE Report	Sean Bradley	Tanya Lewis	Manufacturer Report Number S03-341-252 (15-day initial)	55
5-6-03		FDA Fax	Tanya Lewis	Sean Bradley	Phase 4 Commitments	55
5-6-03		FDA Correspondence	Tanya Lewis	Sean Bradley	Agency's Phase 4 commitment comments.	55
5-2-03		Sponsor FDA E-mail	Sean Bradley	Melody Brown	Responses to FDA Fax received on May 1, 2003.	55
5-2-03	270	SAE Report	Sean Bradley	Tanya Lewis	Manufacturer Report Number S03-341-262 (7-day).	55

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Date	Serial No.	Doc Type	To	From	Description	Location
5-2-03	271	Protocol Amendment / General Correspondence	Richard Pazdur	Renu Vaish	Protocol Amendment: New/Revised Investigators General Correspondence: Transfer of Obligations.	55
5-1-03		FDA Fax	Sean Bradley	Melody Brown	Request for USAN Document	55
5-1-03		FDA Fax	Melody Brown	Sean Bradley	Comments for CMC discussion.	55
4-30-03		FDA Fax	Tanya Lewis	Sean Bradley	NDA Information Request.	55
4-30-03	269	Protocol Amendment	Richard Pazdur	Tanya Lewis	Protocol Amendment: Change in Protocol M34100-026.	55
4-28-03		Sponsor Fax	Sean Bradley	Melody Brown	Response to FDA Fax dated 4-28-03.	55
4-28-03		FDA Correspondence	Tanya Lewis	Sean Bradley	Labeling	55

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4-28-03		FDA Correspondence	Tanya Lewis	Sean Bradley	Labeling	55
4-28-03	267	SAE Report - Fax	Sean Bradley	Tanya Lewis	Notifying Sean of an error in the original cover letter sent on 4-24-03. (S02-341-377)	55
4-28-03	268	SAE Report	Sean Bradley	Tanya Lewis	Manufacturer Report Number S03-341-223.	55
4-24-03	267	SAE Report	Sean Bradley	Tanya Lewis	Manufacturer Report Numbers S03-341-228, S03-341-090, S02-341-377.	55
4-23-03		FDA Fax	Melody Brown	Sean Bradley	NDA - CMC Comments	54
4-23-03		FDA Fax	Tanya Lewis	Sean Bradley	NDA - Clinical Comment	54
4-23-03		FDA Correspondence	Tanya Lewis	Sean Bradley	Revised version of Product Labelin	55

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4-18-03		Form FDA 2656	Melody Brown	FDA	Approved Registration of Drug Establishment / Labeler Code Assignment.	54
4-17-03		FDA Fax	Tanya Lewis	Sean Bradley	Dose modifications during study 025.	54
4-16-03		FDA Fax	Tanya Lewis	Sean Bradley	NDA - Information Request - Study 025	54
4-16-03		FDA Fax	Tanya Lewis	Sean Bradley	Phase 4 commitments from Clinical Pharmacology and Biopharmaceutics division.	54
4-16-03	266	Protocol Amendment	Richard Pazdur	Tanya Lewis	Protocol Amendment: New Protocol M34103-053.	54
4-15-03		FDA Fax	Tanya Lewis	Sean Bradley	Meeting Minutes from December 2, 2002.	54
4-15-03	265	Protocol Amendment	Richard Pazdur	Tanya Lewis	Protocol Amendment: Changes to Protocol and New Investigator Information.	54

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4-11-03		FDA Fax	Melody Brown	Sean Bradley	NDA - CMC Comments/questions.	54
4-10-03		FDA Fax	Tanya Lewis	Sean Bradley	NDA-Clinical Questions	54
4-10-03	264	Protocol Amendment	Richard Pazdur	Tanya Lewis	Protocol Amendment: New Investigator M34102-048 M34102-049 Revised Form FDA 1572: UAB 0280	54
4-8-03		Sponsor FDA E-mail	Sean Bradley	Melody Brown	Questions for discussion - CMC	54
4-8-03	263	SAE Report	Sean Bradley	Tanya Lewis	One follow-up 15-day report. Manufacturer No. S02-341-434	54
4-7-03		FDA Fax	Melody Brown	Sean Bradley	NDA- CMC - Information Request.	54
4-7-03		FDA Correspondence e-mail	Sean Bradley	Melody Brown	Meeting Request - NDA - CMC - answer questions.	54

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Date	Serial No.	Doc Type	To	From	Description	Location
4-3-03		FDA Fax	Tanya Lewis	Sean Bradley	NDA - Information request - CMC	54
4-3-03		FDA Fax	Tanya Lewis	Sean Bradley	NDA - Information Request (additional request) - CMC	54
4-3-03		FDA Fax	Tanya Lewis	Sean Bradley	NDA - Information Request - Patient data - Clinica	54
4-3-03	262	SAE Report	Sean Bradley	Tanya Lewis	Follow-up 15-day safety report. Manufacturer Report No. S02-341-441.	54
4-1-03		FDA Fax	Tanya Lewis	Sean Bradley	Biopharmaceutical comments regarding Special Protocol Assessment Submitted August 2, 2002 Serial # 167.	53
4-1-03		FDA Fax	Tanya Lewis	Sean Bradley	Clinical Questions	53
3-26-03		FDA Fax	Tanya Lewis	Sean Bradley	NDA - PK Patient data questions	53

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3-25-03		Amendment	Dr. Kane	Tanya Lewis	NDA Amendment 8	53
3-25-03	261	Correspondence	Richard Pazdur	Tanya Lewis	Transfer of obligations for various CRO's.	53
3-24-03		FDA Fax	Tanya Lewis	Sean Bradley	Questions regarding our NDA submitted on 12-31-02.	53
3-24-03	259	SAE Reports	Sean Bradley	Tanya Lewis	Two initial 15-day reports, Manufacturer Nos. S03-341-013&S02-341-452. Two follow-up 15-day reports, Manufacturer Nos. S03-341-080&S02-341-363.	53
3-24-03	260	Correspondence	Richard Pazdur	Tanya Lewis	Permission to reference our IND applications and subsequent submissions.	53
3-21-03		FDA Correspondence	Melody Brown	Sean Bradley	FDA has allowed us to use lots from Ash Stevens.	53
3-21-03		FDA Fax	Melody Brown	Electronic Doc. Staff	Fax regarding the electronic NDA submission.	53

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Date	Serial No.	Doc Type	To	From	Description	Location
3-21-03		Correspondence	Jeff Fritsch	Tanya Lewis	Response to letter dated January 15, 2003. Informing Office of Orphan Product that MLNM has submitted NDA.	53
3-20-03	257	Protocol Amendment	Richard Pazdur	Renu Vaish	Protocol Amendment - New Investigator	53
3-20-03	258	Safety Report	Sean Bradley	Tanya Lewis	Two 15-day follow-up reports. Manufacturer nos. S03-341-010 & S02-341-337.	53
3-19-03		FDA Fax	Melody Brown	Sean Bradley	Two lots that were manufactured at Ash-Stevens.	52
3-18-03		Correspondence	Tanya Lewis	Jane Axelrad	User fee	52
3-18-03		Correspondence	Tanya Lewis	Dotti Pease	FDA's filing review of NDA	52
3-18-03	256	Protocol Amendment	Richard Pazdur	Tanya Lewis	Protocol Amendment: Change in Protocol (Expanded Access Protocol	52

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Date	Serial No.	Doc Type	To	From	Description	Location
3-17-03		FDA Fax	Tanya Lewis	Sean Bradley	Safety Pharmacology Studies	52
3-14-03		Correspondence	Chengyi Liang	Melody Brown	Responses to questions - Two lots drug substance tests performed by Ash Stevens.	52
3-14-03		FDA Fax	Tanya Lewis	Sean Bradley	Answers to our questions for upcoming Type A meeting to discuss M34101-039.	52
3-14-03	255	Protocol Amendmet	Richard Pazdur	Renu Vaish	Protocol Amendment - New Investigator	52
3-13-03	254	SAE Reports	Sean Bradley	Tanya Lewis	One 15-day initial S03-341-137 and four 15-day follow-ups S02-341-441, 303, 466 & 235.	52
3-7-03		FDA Fax	Tanya Lewis	Sean Bradley	New drug application - filing review	51
2-28-03		FDA Fax	Tanya Lewis	Sean Bradley	Type A Guidance meeting to discuss 039 study protocol. March 19, 2003 3:30 PM.	51

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2-28-03	251	Correspondence	Richard Pazdur	Tanya Lewis	Authorizing the FDA & Dr. Rasim Gucalp to reference our IND application & subsequent submissions.	51
2-27-03		NDA Amendment	Richard Pazdur	Melody Brown	CMC Information Amendment #004	50
2-27-03	248	Protocol Amendment	Richard Pazdur	Tanya Lewis	Protocol Amendment: Change in protocol.	50
2-27-03	249	Protocol Amendment	Richard Pazdur	Tanya Lewis	New Investigators, New Protocol and Changes in Protocol.	51
2-27-03	250	Correspondence	Richard Pazdur	Tanya Lewis	Authorizing the FDA & Dr. Sven de Vos to reference our IND application & subsequent submissions.	51
2-26-03		FDA Fax	Melody Brown	Sean Bradley	Responses to CMC questions/issues.	50
2-26-03		Correspondence	Melody Brown	Sean Bradley	Teleconference to discuss fax sent to FDA on 21 February 03.	50

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2-25-03	247	SAE Reports	Sean Bradley	Tanya Lewis	SAE Reports, Manufacturer Report Nos. - S03-341-090, S02-341-235, S02-341-397 & S02-341-337.	50
2-21-03		FDA Fax	Tanya Lewis	Sean Bradley	NDA Questions	50
2-21-03		Fax to FDA	Sean Bradley	Julie Batal	Questions for 24 Feb 03 FDA Meeting.	50
2-21-03		Correspondence	Sean Bradey	Julie Batal	Questions for 24 February 03 Meeting	50
2-19-03	244	SAE Reports	Sean Bradley	Tanya Lewis	SAE Reports, Manufacturer Report Nos. - S03-341-080 & S02-341-397.	50
2-19-03	245	Fax to FDA	Sean Bradley	Tanya Lewis	Non-Inferiority Analyses for Study M34101-039	50
2-19-03	246	Fax to FDA	Sean Bradley	Tanya Lewis	Expanded Access Protocol (EAP) Revision.	50

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2-12-03		Correspondence	Sean Bradley	Tanya Lewis	EAP, Post NDA presentation, 039	49
2-12-03	242	SAE Reports	Sean Bradley	Tanya Lewis	SAE Reports, Manufacturer Reports Nos.- S03-341-080, S02-341-386, S02-341-466 & S02-341-377.	49
2-12-03	243	Information Amendment	Richard Pazdur	Tanya Lewis	New Investigator	49
2-10-03		FDA Fax	Tanya Lewis	Sean Bradley	Expanded Access Study 052	49
2-7-03	241	Protocol Amend./Info. Amend.	Richard Pazdur	Renu Vaish	Protocol Amendment: New Investigator Information Amendment: Investigator Brochure	49
2-6-03		Correspondence	Keiko Oishi	Kathleen Meservey	We sent her the following documents: Non-Clinical Overview, Clinical Summary, and Investigator Brochure.	
2-5-03		FDA Fax	Tanya Lewis	Sean Bradley	NDA FDA Meeting scheduled for February 24, 2003 from 4-5:30 pm.	49

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2-3-03		FDA Fax	Tanya Lewis	Sean Bradley	Velcade Presentation.	49
1-31-03		FDA Fax	Tanya Lewis	Sean Bradley	Division of Scientific Investigations.	49
1-31-03		FDA Fax	Tanya Lewis	Sean Bradley	Expanded Access Program	49
1-28-03		Fax	Sean Bradley	Tanya Lewis	Press Release for NDA Submission	49
1-28-03	240	Safety Report	Sean Bradley	Tanya Lewis	One 15-day initial MR # S03-341-025 & one 15-day follow-up MR # S02-341-377.	49
1-27-03		Correspondence	Assoc. Director for	Tanya Lewis	Application refund or user fee ID # 4489.	49
1-27-03	239	Information Amendment	Richard Pazdur	Tanya Lewis	Information Amendment - Investigator Brochure	48

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1-24-03		Correspondence	Jacqueline Cinicola	Janet Woodcock	Informing us of Clinical Trials Data Bank available to the public through the internet.	48
1-23-03		Field Copies	Ellen Madigan	Melody Brown	Field Copies of Clinical and CMC Information Amendment #001	48
1-22-03		Correspondence	Melody Brown	Karen Campbell	Inspection of Cardinal Health	48
1-22-03		Desk Copies	Richard Pazdur	Tanya Lewis	Clinical and CMC Information Amendment #001	48
1-22-03	238	Safety Report	Sean Bradley	Tanya Lewis	Two initial 15-day SAE reports. Manufacturer's Report Nos. S03-341-012 & S03-341-010.	48
1-17-03		Correspondence	Tanya Lewis	Marlene E. Haffner	Orphan Designation Request 02-1630 Notification Letter	48
1-17-03	237	Safety Report	Sean Bradley	Tanya Lewis	7-day initial S03-341-025.	48

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1-16-03		Fax	Tanya Lewis	Jeff Fritsch	Orphan Designation Request 02-1630 Notification Letter	48
1-16-03	236	Treatment Protocol	Richard Pazdur	Tanya Lewis	Clinical Study Protocol M34101-052	48
1-15-03		Fax	Sean Bradley	Colleen Costello	Establishment numbers for NDA #21-602.	47
1-15-03	234	General Correspondence	Richard Pazdur	Tanya Lewis	Authorizing the FDA to reference our IND application and subsequent submissions.	47
1-14-03	235	Safety Reports	Sean Bradley	Tanya Lewis	One 7-day initial S03-341-012, one 15-day initial S02-341-341-466, Two 15-day follow-up reports S02-341-421 & S02-341-373.	47
1-9-03	232	Safety Reports	Sean Bradley	Tanya Lewis	Two follow-up 15-day SAE reports. Manufacturer's Report Nos. S02-341-235 & S02-341-361.	47
1-9-03	233	Protocol Amendment	Richard Pazdur	Tanya Lewis	Change in protocol and New investigator.	47

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Date	Serial No.	Doc Type	To	From	Description	Location
1-6-03		Agency Contact	Rachel Pratt/Tanya	Sean Bradley	NDA Submission binder color.	47
1-3-03		Field Copy Letter	Ms. Ellen Madigan	Melody Brown	Field copy of NDA and registered establishment information.	47
1-2-03		Submission of Form	Information Mngmnt.	Melody Brown	Submission of Form 2656	47
1-2-03		Agency Contact	Ms. Ellen Madigan	Julie Batal	Disposition of Field Copies of PS-341 NDA	47
12-31-02	231	Meeting Minutes	Sean Bradley	Tanya Lewis	EOP2 Meeting minutes from meeting on December 2, 2002.	47
12-30-02		General Correspondence	Sean Bradley	Melody Brown	Follow-up to IND Amendment with Establishment Information.	47
12-30-02	230	Safety Reports	Sean Bradley	Tanya Lewis	15-day Manufacturer's Report Nos. S02-341-441 & S02-341-386.	47

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12-26-02	229	Safety Reports	Sean Bradley	Tanya Lewis	15-day Manufacturer's Report Nos. S02-341-434, S02-341-435 & S02-341-440	47
12-24-02		Fax -User Fee ID #	Tanya Lewis		FDA Fax - User Fee ID #	47
12-24-02	227	General Correspondence	Richard Pazdur	Tanya Lewis	Authorizing FDA to reference our Investigational New Drug Application and subsequent submissions.	47
12-24-02	228	Expanded Access Program	Richard Pazdur	Tanya Lewis	Expanded Access Program: Protocol M34101-052 Synopsis	47
12-20-02	226	Response to Request for Information	Richard Pazdur	Melody Brown	CMC - Response to Request for Information	47
12-19-02	225	Safety Report	Sean Bradley	Tanya Lewis	15-day Manufacturer's Report No. S02-341-419	47
12-17-02	224	Safety Report	Sean Bradley	Tanya Lewis	15-day Manufacturer's Report No. S02-341-415	47

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12-16-02	221	Safety Report	Sean Bradley	Tanya Lewis	7-day Manufacturer's Report No. S02-341-421	45
12-16-02	222	Response to Inform. Request	Richard Pazdur	Melody Brown	Reaponse to information request: CMC	45
12-16-02	223	Protocol Amendment	Richard Pazdur	Renu Vaish	New Investigator and Change in Protocol 034 & 049	46
12-12-02		FDA Fax	Tanya Lewis	Sean Bradley	Responses to our questions pertaining to the upcoming NDA submission - Clinical	45
12-12-02		FDA Fax	Meldody Brown	Sean Bradley	CMC Teleconference	45
12-12-02		Agency Contact	Melody Brown	S. Bradley & R.	FDA's responses to Action Items from the Velcade pre-NDA CMC Meeting (November 5, 2002)	45
12-11-02		Orphan Drug Meeting			IP/Orphan Drug Meeting on Wednesday, December 11, 2002 - Location OBW 5D	

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12-9-02		General Correspondence	Sean Bradley	Renu Vaish	Physician information for patient on study protocol 029.	45
12-9-02	220	Request for Information	Richard Pazdur	Melody Brown	Response to request for information; CMC	45
12-6-02		General Correspondence	Tanya Lewis	Jeffrey Fritsch	Letter acknowledging receipt of orphan designation application.	45
12-6-02		Slide Presentation	Sean Bradley	Tanya Lewis	Dr. Schenkein's slide presentation. Teleconference meeting request.	45
12-6-02	219	General Correspondence	Sean Bradley	Renu Vaish	Letter regarding protocol exemption for 341 and carboplatin.	45
12-5-02	217	Sponsor Fax	Sean Bradley	Tanya Lewis	Teleconference Meeting Request	45
12-5-02	218	Safety Report	Sean Bradley	Tanya Lewis	One 7-day and one 15-day initial IND Safety Reports - Manufacturer Report Nos. S02-341-415 & S02-341-397.	45

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12-4-02		MLNM Press Release	Sean Bradley	Robert Pietrusko	Fax MLNM Press Release	45
12-3-02		Sponsor Fax	Peter Bross	Robert Pietrusko	ASH Abstracts and Symposium Info.	45
12-3-02	216	Safety Report	Sean Bradley	Tanya Lewis	15-day initial Safety Report - Manufacturer Report No. S02-341-377	45
11-26-02	209	Annual Report	Richard Pazdur	Renu Vaish	Annual Report for reporting period of September 1, 2001 through August 30, 2002.	44
11-26-02	213	Safety Reports	Sean Bradley	Renu Vaish	Two 7-day and one 15-day Initial IND Safety Reports - Manufacturer Report Nos. S02-341-377, S02-341-397, and S02-341-361.	44
11-26-02	214	Protocol Amendment	Richard Pazdur	Tanya Lewis	Protocol Amendment: New Investigator	44
11-26-02	215	Safety Reports	Sean Bradley	Renu Vaish	Three 15-day IND Safety Reports - Manufacturer Report Nos. - S02-341-373, S02-341-386 and S02-341-303.	44

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11-25-02		Agency Fax	Tanya Lewis	Sean Bradley	Pre-NDA Meeting - FDA's response to question for discussion.	43
11-22-02	212	Safety Report	Sean Bradley	Tanya Lewis	7-day IND Safety Report - Manufacturer Report No. S02-341-373.	43
11-19-02	211	Safety Report	Sean Bradley	Renu Vaish	15-day IND Safety Reports - Manufacturer Report Nos. S02-341-363, S02-341-225 (Faxed & Fed Exed)	43
11-15-02		Agency Fax	Tanya Lewis	Sean Bradley	Copy of the Fast-Track Designation letter originally issued May 2002.	43
11-15-02	210	Safety Report	Sean Bradley	Tanya Lewis	7-day Initial Faxed Safety Report Manufacturer Report No. S02-341-361	43
11-14-02	207	Information Amendment	Richard Pazdur	Melody Brown	Inforamtion Amendment: Chemistry, Manufacturing, and Control; Pharmacology - Toxicology	43
11-14-02	208	Protocol Amendment Information Amendment	Richard Pazdur	Renu Vaish	Information Amendment - Clinical Protocol Amendment - New Investigator / Change in Protocol	43

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11-13-02	206	Safety Reports	Sean Bradley	Tanya Lewis	15-day IND Safety Report - Manufacturer Report Nos. S02-341-353 Initial and S02-341-233 follow-up.	43
11-11-02	205	Safety Report	Sean Bradley	Tanya Lewis	15-day IND Safety Report - Manufacturer Report No. S02-341-344.	42
11-8-02	204	Briefing Document	Richard Pazdur	Tanya Lewis	End of Phase II Briefing Document.	42
11-4-02	203	Safety Reports	Sean Bradley	Renu Vaish	15-day IND Safety Reports - Manufacturer Report Nos. S02-341-195, S02-341-337, S02-341-174	42
11-1-02		Sponsor Fax	Sean Bradley	Melody Brown	Sponsor responses of FDA comments - CMC Meeting Questions.	42
11-1-02	202	Information Amendment	Richard Pazdur	Tanya Lewis	Information Amendment: Request for Clinical, Statistical, and IT Input	42
10-31-02	201	Information Amendment	Richard Pazdur	Tanya Lewis	Information Amendment: Request for Non-Clinical Input.	42

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10-30-02	200	Fax	Sean Bradley	Tanya Lewis	Teleconference Meeting Minutes September 27, 2002.	42
10-29-02		General Correspondence	Melody Brown	Sean Bradley	E-mail confirming Pre-NDA CMC Meeting on November 5, 2002.	42
10-28-02	199	Safety Report	Sean Bradley	Tanya Lewis	15-day IND Safety Report - Initial Manufacturer's Report No. S02-341-319	42
10-23-02	197	Safety Report	Sean Bradley	Tanya Lewis	15-day IND Safety Report - Initial Manufacturer's Report No. S02-341-312	41
10-22-02	195	Safety Report	Sean Bradley	Tanya Lewis	15-day IND Safety Report - Initial Manufacturer's Report No. S02-341-303	41
10-22-02	196	Gerneral Correspondence	Richard Pazdur	Tanya Lewis	Authorize FDA to reference MLNM's IND & subsequent submissions for Velcade - submitted to the Agency by Dr. Heinz-Josef Lenz.	41
10-22-02	198	Gerneral Correspondence	Richard Pazdur	Tanya Lewis	Authorize FDA to reference MLNM's IND & subsequent submissions for Velcade - submitted to the Agency by Helen K. Chew, M.D.	41

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10-17-02	194	Protocol Amendment	Richard Pazdur	Renu Vaish	Protocol Amendment - New Protocol: M34102-048.	41
10-16-02	193	Safety Report	Sean Bradley	Tanya Lewis	15-day IND Safety Report - Initial Manufacturer's Report No. S02-341-235	41
10-15-02	191	Safety Report	Sean Bradley	Tanya Lewis	15-DAY IND SAFETY REPORT - FOLLOW-UP Manufacturer's Report No. S02-341-213	41
10-15-02	192	Pre-NDA CMC Briefing Document	Richard Pazdur	Melody Brown	Briefing Docment for Pre-NDA CMC Meeting	41
10-8-02	190	Gerneral Correspondence	Richard Pazdur	Tanya Lewis	Authorize FDA to reference MLNM's IND & subsequent submissions for Velcade - submitted to the Agency by Dr. Jeffrey A. Sosman.	41
10-7-02	189	Safety Report	Sean Bradley	Tanya Lewis	Initial 15-day Safety Report. Manufacturer's Report No. S02-341-083.	41
10-4-02	188	Safety Report	Sean Bradley	Tanya Lewis	Initial 15-day Safety Report. Manufacturer's Report No. S02-341-243. Response to initial fax 7-day report submitted on 20 Sept 02	41

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10-3-02	187	Safety Report	Sean Bradley	Tanya Lewis	Initial 15-day Safety Report. Manufacturer's Report # S02-341-025.	41
10-2-02	186	Protocol Amendment	Richard Pazdur	Renu Vaish	Serial # 186 - Protocol Amendment - New Protocol: M34101-049.	41
9-30-02		Orphan Drug Application	Orphan Products Dev.	Tanya Lewis	Orphan Drug Application . Requesting orphan designation for Velcade to treat multiple myeloma.	40
9-30-02	185	Protocol Amendment NI	Richard Pazdur	Tanya Lewis	Serial #185 - Protocol Amendment: New Investigator Information	39
9-27-02	183	Safety Report	Sean Bradley	Tanya Lewis	Follow-up 15-day Safety Report. Manufacturer's Report No. S02-341-093. Patient experienced Drug eruption NOS and pain in limb.	38
9-27-02	184	Safety Report	Sean Bradley	Tanya Lewis	Initial 15-day Safety Report. Manufacturer's Report No. S02-341-249. Fecal impaction and Herpes zoster.	38
9-26-02	182	Safety Report	Sean Bradley	Tanya Lewis	Initial 7-day Safety Report. Manufacturer's Report No. S02-341-265. Respiratory distress, Renal failure NOS, Metabolic encephalopathy NOS, Convulsions NOS and Disease progression resulting in death.	38

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9-20-02	180	7 Day Safety Report	Sean Bradley	Tanya Lewis	Initial 7-day Safety Report. Manufacturer's Report No. S02-341-243. Renal Failure resulting in death.	38
9-20-02	181	Type B Meeting Request	Richard Pazdur	Melody Brown		38
9-19-02		Fax from FDA	Renu Vaish	Sean Bradley	PS-341 Meeting Request - Regarding: August 2, 2002 request for Special Protocol Assessment	38
9-19-02	179	FDA Correspondence	Sean Bradley	Tanya Lewis	IND 56,515 Serial #179 Type A Meeting Request	38
9-18-02	178	Fax to FDA	Sean Bradley	Tanya Lewis	EOP II Meeting Minutes Serial #178 FAXED ON 9-17-02	38
9-17-02	177	Safety Report	Sean Bradley	Tanya Lewis	Initial 15-day Safety Report Serial #177. Report No. S02-341-233	38
9-10-02	176	Safety Report	Sean Bradley	Tanya Lewis	Follow-up 15-day Safety Report Serial # 176. Report No. S02-341-174	38

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9-3-02	175	Safety Report	Sean Bradley	Renu Vaish	Initial 15-day Safety Report Serial #175. Report No. S02-341-225	38
8-30-02		FDA Correspondence	Tanya Lewis	Sean Bradley	Fax regarding Velcade/EOP2 Meeting. Answers to questions in Briefing Document.	38
8-26-02	174	Special Protocol Assessment Responses	Richard Pazdur	Tanya Lewis	Responses to comments raised during Special Protocol Assessment	37
8-23-02	173/171	Safety Report	Sean Bradley	Tanya Lewis	Initial 15-day Safety Report Serials # 171 (It is supposed to be serial #173). Manufacturer's Report No. S02-341-218	36
8-21-02	172	Safety Report	Sean Bradley	Tanya Lewis	Follow-up 15-day Safety Report Serial #172 Manufacturer's Report No. S02-341-046	36
8-16-02	171	Safety Report	Sean Bradley	Tanya Lewis	Initial 15-day Safety Report Serial # 171 Manufacturer's Report No. S02-341-213	36
8-13-02	170	Briefing Document	Richard Pazdur	Tanya Lewis	End of Phase II briefing document	36

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8-9-02	169	General Correspondence	Richard Pazdur	Tanya Lewis	Authorize FDA to reference MLNM's IND & subsequent submissions for Velcade - submitted to the Agency by H.Scher.	36
8-7-02	168	Protocol Amendment	Richard Pazdur	Renu Vaish	Change in protocol & New investigator	36
8-5-02	166	Protocol Amendment: New Investigator	Richard Pazdur	Tanya Lewis	New Investigators added to 039 and 040	35
8-2-02	167	Special Protocol Assessment	Richard Pazdur	Renu Vaish	Special Protocol Assessment / Phase III Clinical Study Protocol Serial No. 167	34
8-1-02		Telephone report	Sean Bradley	Renu Vaish	Confirmatory voicemail that Phase 3 Pancreatic Protocol will be submitted for Special Protocol Assessment tomorrow, ie. 02Aug02.	36
7-31-02	165	General Correspondence	Sean Bradley	Tanya Lewis	Correspondence informing Agency of PS-341 tradename.	34
7-25-02		Telephone report	Sean Bradley	Renu Vaish	Returned phone message regarding cancellation of meeting.	34

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7-25-02		Telephone report	Sean Bradley	Renu Vaish	Confirmation of responses to questions in the briefing document.	34
7-24-02		Telephone report	Sean Bradley	Renu Vaish	Briefing document & contact info for Washington trip.	34
7-24-02		Fax from FDA	Renu Vaish	Sean Bradley	Velcade (bortezomib) / meeting request	34
7-24-02	164	Information amendment	Richard Pazdur	Melody Brown	Information Amendment: Chemistry, Manufacturing, and Control	34
7-19-02	162	General Correspondence	Richard Pazdur	Tanya Lewis	Authorize FDA to reference MLNM's IND & subsequent submissions for Velcade - submitted to the Agency by S.Jagannath.	34
7-19-02	163	General Correspondence	Richard Pazdur	Tanya Lewis	Authorize FDA to reference MLNM's IND & subsequent submissions for Velcade - submitted to the Agency by H.Scher.	34
7-11-02	161	IND 56,515	Sean Bradley	Tanya Lewis	15-Day IND Safety Report - Initial Manufacturer's Report No. S02-341-174 15-Day IND Safety Report - Follow-up Manufacturer's Report No. S02-341-046	34

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7-8-02		Telephone report	Sean Bradley	Renu Vaish	Company's perspective in moving forward with this drug, in consideration of the neurotoxicities seen. (per Dr. Pazdur)	34
7-8-02		Telephone report	Sean Bradley	Renu Vaish	Questions for preclinical group.	34
7-3-02	159	IND 56,515 Briefing Document	Richard Pazdur	Renu Vaish	Briefing Document for Type B Meeting	33
6-26-02		Fax from FDA	Tanya Lewis	Sean Bradley	Clinical Benefit Plan Summary	33
6-17-02	158	General Correspondence	Richard Pazdur	Bernadette Bowen	Cross reference for MD Anderson Cancer Center, Dr. Pedro Ramirez	33
6-12-02		Fax to FDA	Sean Bradley	Jackie Cinicola	Fax regarding follow-up from SPA meeting Clinical Study protocol M34101-039 May 28, 2002	33
6-12-02		Fax to FDA	Sean Bradley	Tanya Lewis	IND 56,515 Follow-up from SPA Meeting Clinical Study Protocol M34101-039 May 28, 2002	33

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6-12-02	156	Protocol Amendment	Richard Pazdur, MD	Renu Vaish	Change in Protocol M34101-028 and New Investigator Information for M34101-034/Dr. Marianna Koczwas.	33
6-12-02	157	Follow-up Safety Report	Sean Bradley	Bernadette Bowen	15-day IND Safety Report-Follow-up Manufacturer's Report NO. S02-341-088	33
6-11-02	155	15-day Initial Safety Report	Sean Bradley	Bernadette Bowen	15-day IND Safety Report-Initial Manufacturer's Report NO. S02-341-046	33
6-7-02		Fax from FDA	Renu Vaish	Sean Bradley	Meeting request	32
6-6-02		Fax from FDA	Tanya Lewis, MS	Sean Bradley	Meeting request notification for End-of-Phase 2 meeting September 4, 2002.	32
5-31-02	154	General Correspondence	Richard Pazdur	Renu Vaish	Request for Type B meeting for pancreatic cancer.	32
5-30-02		Fax to FDA	Sean Bradley	Jackie Cinicola	IND 56,515 BioPharm and Pharmacology/Toxicology Responses (25 January 2002)	32

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5-30-02	152	IND #56,515	Richard Pazdur	Jackie Cinicola	Protocol Amendment - Change in Protocol Independent Review Committee Charter Statistical Analysis Plan	32
5-30-02	153	FDA Correspondence	Richard Pazdur	Jackie Cinicola	IND #56,515 Request for Type B Formal Meeting End of Phase 2 Meeting	32
5-24-02		FDA Correspondence	Jackie Cinicola	Richard Pazdur, MD	Fast Track designation letter	32
5-20-02	151	Follow-up Safety Report	Sean Bradley	Bernadette Bowen	15-day IND Safety Report-Follow-up Manufacturer's Report NO. S01-341-075	32
5-17-02		fax to FDA	Sean Bradley	Jackie Cinicola	attachment of briefing document for SPA meeting scheduled for 5/28. 8 hard copies is being submitted to the IND	31
5-17-02	150	Special Protocol Assessment Meeting Briefing Document	Richard Pazdur	Jackie Cinicola	IND 56, 515 Special Protocol Assessment Meeting 28 May 2002 12:30 p.m. Review of Clinical Study Protocol M34101-039	31
5-16-02		Fax to FDA	Sean Bradley	Jacqueline Cinicola	Fax to FDA ps341 response to SPA comments	31

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5-16-02	149	Protocol Amendment	Richard Pazdur	Tanya Lewis	Change in Protocol M34100-027. Addition of dose cohorts, increase maximum sample size from 50 to 60	31
5-15-02		email to FDA	Jackie Cinicola	Sean Bradley	no additional comments on Protocol-039. please submit protocol 040 for review.	31
5-15-02	148	Protocol Amendment: Change in Protocol	Richard Pazdur	Bernadette Bowen	Changes in Protocol No. M34101-034, new investigators	31
5-13-02		email to FDA	Sean Bradley	Jackie Cinicola	Discussion of SPA for clinical protocol M34101-039	31
5-10-02		Record of Regulatory Agency Contact	Sean Bradley	Jackie Cinicola	Special Protocol Assessment regarding the status of the clinical benefit questions	31
5-10-02		Fax from FDA	Melody Brown	Sean Bradley	IND 56,515 Special Protocol Assessment for protocol M34101-040	31
5-7-02		Record of Regulatory Agency Contact	Sean Bradley	Jackie Cinicola	Special Protocol Assessment to determine the status of the Agency's questions regarding clinical benefit	31

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Date	Serial No.	Doc Type	To	From	Description	Location
5-7-02	147	15-DAY IND SAFETY REPORT - FOLLOW-UP REPORT	Sean Bradley	Bernadette Bowen	Mnfr's Report S00-341-044 of myocardial infarction, cardiac failure congestive, suicidal ideation, bacterial infection NOS, Patient #13, enrolled in Protocol #LCCC 9834(Orlowski)	31
5-6-02		Record of Regulatory Agency Contact	Sean Bradley	Jackie Cinicola	Type A meeting regarding SPA M34101-039; fax listed the FDA atendeees	31
5-6-02		Meeting Minutes	Millennium	Chaengyi Liang	End of Phase II CMC, seeking division input on CMC development activities	31
5-6-02		Fax from FDA	Melody Brown	Sean Bradley	copy of minutes for April 9 2002 CMC meeting between representitives from Millennium and FDA	31
5-6-02		Fax from FDA	Jacqueline Cinicola	Sean Bradley	fax serves as a notice that the april 26, 2002 request for type A regarding drug ps341 has been granted	31
5-2-02		Record of Regulatory Agency Contact	Sean Bradley	Jacqueline Cinicola	Verification of meeting for May 28 @ 12:30 pm, questioned status of clinical benefit comments	31
4-29-02		Record of Regulatory Agency Contact	Sean Bradley	Jackie Cinicola	Confirmed the request for a Type a meeeting, determine the status of the clinical benefit comments.	30

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Date	Serial No.	Doc Type	To	From	Description	Location
4-26-02	146	Request for Meeting	Richard Pazdur M.D	Jackie Cinicola	Request for Type A Formal Meeting Special Protocol Assessment (SPA) - Clinical Protocol M34101-039, 2 April 2002, Sponsor Response to SPA, Serial #141, 14 March 2002 R. Pazdur, Response to	30
4-24-02		Fax from FDA	Jacqueline Cinicola	Sean Bradley	comments concerning duration of therapy and alternative therapy sent in reference to your request for Fast Track Designation	30
4-17-02		Special Protocol Assessment Meeting Briefing Document	Richard Pazdur	Jackie Cinicola	Special Protocol Assessment Meeting to address the Agency's recent comments regarding duration of therapy time to progression and clinical benefit analysis	
4-17-02	145	Protocol Amendment: Change in Protocol	Richard Pazdur M.D	Jackie Cinicola	New Protocol: Changes in Protocols (M34101-040, M34100-026 and M34101-033)	
4-17-02	145	New Protocol 040, 026, 033	Richard Pazdur	Bernadette Bowen	Changes to protocols M34101-040, M34100-026, M34101-033	
4-15-02	144	15-DAY IND SAFETY REPORT - INITIAL REPORT	Sean Bradley	Jackie Cinicola	Mnfr's Report S02-341-093 of vesicular rash Patient #011-005(EB), enrolled in Protocol #M34100-025(Siegel)	29
4-15-02	144	15-day IND safety report	Sean Bradley	Bernadette Bowen	submitting an initial 15-day IND safety report of rash vesicular and pain in limb. Report is for patient no. 011-005 enrolled in Clinical study protocol M341-025	

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Date	Serial No.	Doc Type	To	From	Description	Location
4-5-02	143	15-DAY IND SAFETY REPORT - INITIAL REPORT	Sean Bradley	Jackie Cinicola	Mnfr's Report S02-341-088 of failure to thrive Patient #011-004(JAE), enrolled in Protocol #M34100-025(Siegel)	29
4-5-02	143	15-day IND Safety Report	Sean Bradley	Bernadette Bowen	submitting an initial 15-day IND safety report of failur eto thrive of unclear etiology for patient no. 011-004, enrolled in Clinical Study Protocol No. M34100-025	29
4-4-02		Record of Regulatory Agency Contact	Sean Bradley	Jackie Cinicola	Incorrect document sent in error. Section 11.1 was sent in place of Section 11.11. Mr. Bradley requested that section 11.11 be faxed to agency	29
4-4-02		Fax to FDA	Sean Bradley	Jackie Cinicola	Attachment of Protocol M34101-039 section 11.7 was faxed	29
4-3-02		Fax to FDA	Sean Bradley	Jackie Cinicola	confirming the receipt of the fax outlining the response to special protocol assessment return	
4-3-02		Fax to FDA	Sean Bradley	Jackie Cinicola	attached sections of protocol 341-039 sections 3.4.7.1, 3.6.2.3, 6.5, 11.1	
4-3-02		General Correspondence	Richard Pazdur	Melody Brown	General Correspondence letter presenting new info on a synthesis related impurity in PS341	29

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Date	Serial No.	Doc Type	To	From	Description	Location
4-3-02		Fax to FDA	Sean Bradley	Jacqueline Cinicola	Fax attaching various sections from Clinical Study Protocol M34101-039: 3.4.7.1 Permitted Medications and Supportive Therapies	29
4-3-02		Record of Regulatory Agency Contact	Sean Bradley	Jackie Cinicola	confirmation of fax	29
4-3-02	141	General Correspondence	Richard Pazdure, MD	Melody Brown	General Correspondence addressing the Impurity F Biologic Activity	29
4-3-02	142	Fax to FDA	Sean Bradley	Melody Brown	Fax noting the copy of submission was sent via FedEx	29
4-2-02	141	Fax to FDA	Sean Bradley	Jacqueline Cinicola	Response tp Special Protocol Assessment (R. Pazdur 3-14-02) Request for Information	29
4-2-02	142	Response to Special Protocol Assessment	Richard Pazdur	Jackie Cinicola	Response to Special Protocol Assessment (R. Pazdur, 14 March 2002, Serial #124)	29
3-23-02		MPI Fax to FDA	Sean Bradley	Jackie Cinicola	Request for information	29

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Date	Serial No.	Doc Type	To	From	Description	Location
3-22-02		Email from FDA	Melody Brown	Sean Bradley	PS-341 End of Phase II CMC List of CMC questions and List of Attendees	29
3-22-02	139	Protocol Amendment: Change in Protocol	Richard Pazdure, MD	Jackie Cinicola	Submitting Amendment 3 for both 027 and 028.	29
3-22-02	140	15-DAY IND SAFETY REPORT - FOLLOW-UP	Sean Bradley	Jackie Cinicola	15-DAY IND SAFETY REPORT - 2nd FOLLOW-UP to Manufacturer's Report No. S01-341-081	29
3-14-02	137	Protocol Amendment: New Investigators	Richard Pazdure, MD	Meri Bloom	PROTOCOL AMENDMENT: New Investigators for 029, Bart Barlogie, MD and James R. Berenson, MD. Revised 1572 for 026, John G. Gribben, MD	28
3-14-02	138	15-DAY IND SAFETY REPORT - FOLLOW-UP	Sean Bradley	Meri Bloom	15-DAY IND SAFETY REPORT - 2nd FOLLOW-UP to Manufacturer's Report No. S02-341-010	28
3-13-02	136	End of Phase II CMC Briefing Document	Richard Pazdur, MD	Melody Brown	Briefing Package w/questions for End of Phase II CMC Meeting scheduled for April 9, 2002	28
3-11-02	135	General Correspondence	Richard Pazdur, MD	Meri Bloom	Letter to authorize FDA to reference MPI's IND and subsequent sub by U.T. M.D. Anderson Cancer Center, Department of Lymphoma & Myeloma for the same product under Andre Goy M.D.	28

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Date	Serial No.	Doc Type	To	From	Description	Location
3-7-02		General Correspondence	Jackie Cinicola	Terry Toigo	Clinical Trial Data Bank requirements applicable to the 039 protocol submitted as serial number 124, January 25, 2002	28
2-27-02	132	15 Day IND Follow Up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report #S02-341-010 of Pyrexia, Renal Impairment NOS and Lung Nodule, Patient No. 004-001(ECB), enrolled in Protocol # M34100-025(Alsina)	28
2-27-02	133	General Correspondence	Richard Pazdur, MD	Meri Bloom	Letter to authorize FDA to reference MPI's IND and subsequent sub by Baylor College of Medicine for the same product under Dr. Robert J. Amato	28
2-27-02	134	General Correspondence	Richard Pazdur, MD	Meri Bloom	Letter to authorize FDA to reference MPI's IND and subsequent sub by Cedars-Sinai Medical Center for the same product under Dr. James Berenson	28
2-25-02		FDA Fax	Melody Brown	Sean Bradley	Fax scheduling CMC meeting for April 9, 2002; request to send attendees list and request to submit 5 bounding copies of the meeting package by March 11, 2002	28
2-21-02	131	15 Day IND Follow Up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report #S01-341-081 of Pleuritic pain, Patient No. 013-108(GHS), enrolled in Protocol #M34101-028(Ryan)	28
2-15-02		Record of Regulatory Agency Contact	Sean Bradley	Jackie Cinicola	M34101-039 Special Protocol Assessment- Increase in Sample Size	

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Date	Serial No.	Doc Type	To	From	Description	Location
2-13-02	130	Formal Request for Type B Meeting	Richard Pazdur, MD	Melody Brown	Submission: Formal Request for Type B Meeting (End-of-Phase II CMC Meeting)	28
2-11-02	129	Protocol Amendment New Protocol	Richard Pazdur, MD	Meri Bloom	New Protocol: #M34101-040 entitled: "An International, Non-Comparative, Open-Label Study of PS-341 Administered to Patients with Multiple Myeloma Who Experienced Relapsed or Progressive	28
2-6-02	128	15 Day IND Follow Up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-068 of Chest tightness, Dyspnoea NOS, Postural hypotension and Herpes zoster, Patient No. 008-002(DRP), enrolled in Protocol #M34100-025(Orlowski)	28
2-4-02	127	15 Day IND Follow Up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S00-341-044 of Myocardial Infarction, Cardiac failure congestive, Depression suicidal and Bacteraemia, Patient No. 13, enrolled in Protocol #LCCC 9834(Orlowski)	28
2-1-02		General Correspondence	Jacqueline Cinicola	Dottie Pease	Aknowledgement receipt for the January 25, 2001, serial no. 124 request for a special protocol assessment	28
1-31-02		Record of Regulatory Agency Contact	Sean Bradley	Jackie Cinicola	New Medical Reviewer Assigned- Peter Bross, M.D	
1-25-02	123	15 Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S02-341-010 of Pneumonia fungal NOS and Renal Impairment NOS, Patient #004-001(ECB), enrolled in Protocol #M34100-025(Alsina)	27

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Date	Serial No.	Doc Type	To	From	Description	Location
1-25-02	124	Request for Special Protocol Assessment	Sean Bradley	Jackie Cinicola	Request for Special Protocol Assessment; Review of Clinical Study Protocol M34101-039; Sponsor Action Item; End-of-Phase 2 Meeting (December 7, 2001)	27
1-25-02	125	Response to Agency Request for Information	Sean Bradley	Jackie Cinicola	Response to Request for Information; Sponsor Action Items; End-of-Phase 2 (EOP2) Meeting (December 7, 2001)	28
1-25-02	126	Request for Fast Track Designation	Richard Pazdur, MD	Jackie Cinicola	Request for Fast Track Designation following a Non-Designation Determination	28
1-18-02	122	Protocol Amendment: New Investigators & Revised Forms FDA 1572	Richard Pazdur, MD	Meri Bloom	024: Richardson; 027: Ryan; 028: Ryan, 029: Alsina, Irwin, Jagannath, Richardson and Kuter; 033: Dreicer and Roth; 034: Gandara and Lara	27
1-14-02	121	15 Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-093 of Hyperuricaemia, Patient #009-010(JR), enrolled in protocol #M34100-025(Richardson)	26
1-9-02	120	15 Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-080 of Syncope and Facial palsy, Patient #002-014(AHL), enrolled in protocol #M34100-025(Barlogie)	26
1-7-02	119	General Correspondence	Richard Pazdur, MD	Meri Bloom	Letter to authorize FDA to reference MPI's IND and subsequent sub by the Arkansas Cancer Research Center at the University of Arkansas for Medical Sciences for the same product	26

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Date	Serial No.	Doc Type	To	From	Description	Location
1-4-02	117	15 Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-075 of Weakness, Diarrhoea NOS and Cardio-respiratory arrest, Patient #003-027(BPB), enrolled in 025(Vescio)	26
1-4-02	118	15 Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-068 of Chest tightness, Dyspnoea NOS, Postural hypo and Herpes zoster, Patient #008-002(DRP), enrolled in 025(Orlowski)	26
12-28-01		Record of Regulatory Agency Contact	Sean Bradley	Jackie Cinicola	Identification of Medical Officer and follow-up from EOP-2 meeting. When new medical officer is assigned Sean will let us know.	
12-21-01	116	Response to FDA Request for Information	Sean Bradley	Meri Bloom	Response to question: "Please clarify whether gross hemolysis was observed on 10-9-01. If so, why was the patient treated with PS-341 in the presence of gross hemolysis?"	26
12-20-01	115	End of Phase 2 Meeting Minutes	Sean Bradley	Jacqueline Cinicola	End of Phase 2 Meeting Minutes held on December 7, 2001 with copy of slides presented during meeting attached; acknowledgement of Receipt of Agency Minutes	26
12-19-01		FDA Fax	Meri Bloom	Sean Bradley, CSO	Meeting minutes from December 7, 2001 End of Phase II meeting.	26
12-17-01	114	15 Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-089 of Neutropenic sepsis, Patient #003-030(BJB), enrolled in protocol #M34100-025(Berenson)	26

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Date	Serial No.	Doc Type	To	From	Description	Location
12-14-01	113	Information Amendment: Investigator's Brochure	Richard Pazdur, MD	Meri Bloom	Investigator's Brochure Version 5.0 - with Section 4 and Section 5 updated	26
12-13-01	111	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-009 of hypercalcaemia, Patient #17(NGI) enrolled in protocol #LCCC9834(Orlowski)	26
12-13-01	112	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-061 of respiratory failure (exc neonatal), Patient #005-001(SJM), enrolled in protocol #M34100-025(Hussein)	26
12-12-01	107	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S00-341-034 of Hyponatraemia, Patient # 10 enrolled in protocol #LCCC 9834(Orlowski)	26
12-12-01	108	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S00-341-044 of Myocardial infarction, cardiac failure congestive, Depression suicidal, Bacteraemia, Patient #13 enrolled in protocol #LCCC 9834(Orlowski)	26
12-12-01	109	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-093 of Hyperuricaemia, Patient #009-010(JR) enrolled in protocol #M34100-025(Richardson)	26
12-12-01	110	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S00-341-028 of serum sickness and Dyspnoea NOS, Patient #06, enrolled in protocol #LCCC 9834(Orlowski)	26

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Date	Serial No.	Doc Type	To	From	Description	Location
12-10-01	104	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-064 of Ataxia NEC, Weakness and Peripheral neuropathy Nec, Patient # 014-002 enrolled in protocol #M34100-024(Irwin)	26
12-7-01	105	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-058 of Pancreatitis NOS, Patient #005-003(ARW) enrolled in protocol #M34100-025(Hussein)	26
12-7-01	106	Protocol Amendment: New Investigators	Richard Pazdur, MD	Meri Bloom	024 William I. Bensinger, MD; Asher Chanan-Khan, MD; Michael W. Schuster, MD Gordan Srkalovic, MD 025 Vincent S. Rajkumar, MD and 026 1572 for Ian W. Flinn	26
12-6-01		Record of Regulatory Agency Contact	Sean Bradley	Jackie Cinicola	Ps341 end of pahse 2 meeting, S.Bradley confirmed the receipt of updated list of MPI attendees	
12-3-01		email to FDA	Sean Bradley	Jackie Cinicola	attachment of updated list of Millennium participants for end of phase 2 meeting	
11-23-01		Record of Regulatory Agency Contact	Regulatory file	Jackie Cinicola	prepare to send updated list of MPI participanta to Agency. Reorganize end of phase 2 slide presentation	
11-21-01		FDA Fax	Meri Bloom	Sean Bradley	Response to Fast track request made September 20, 2001 submitted under serial no. 073	25

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Date	Serial No.	Doc Type	To	From	Description	Location
11-20-01	102	15 Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-093 of Hyperuricaemia, Patient #009-010 (JR) enrolled in Protocol #M34100-025(Richardson)	25
11-20-01	103	15 Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-090 of Vomiting NOS, Hyperuricaemia, Neutropenia and Diarrhoea NOS, Patient # 006-002 (JV) enrolled in protocol #M34100-025(Jagannath)	25
11-16-01	097	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S00-341-002 of Pyrexia, Neutropenia and Leukopenia NOS, Patient #27(WW) enrolled in protocol #98-104(Aghajanian)	25
11-16-01	098	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S00-341-04 of Myocardial Infarction, Cardiac failure cong, depression suicidal, Bacteraemia and Haemoglobin decreased, Patient # 13 enrolled in protocol #LCCC 9834(Orlowski)	25
11-16-01	099	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S00-341-033 of peripheral neuropathy, Patient # 35 enrolled in protocol #98-104(Aghajanian)	25
11-16-01	100	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S00-341-011 of nasopharyngeal cancer, Patient # 31 enrolled in protocol #98-104 (Aghajanian)	25
11-16-01	101	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-075 of Weakness, Diarrhoea NOS and cardio-respiratory arrest, Patient # 003-027 enrolled in protocol #M34100-025(Vescio)	25

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Date	Serial No.	Doc Type	To	From	Description	Location
11-12-01	095	Safety Report - Laboratory Animals	Sean Bradley	Meri Bloom	Official regulatory notification for the observance of pos. results from ongoing genotox. assay with PS-341	25
11-12-01	096	15 Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-063 of Confusion and Hyponatremia, Patient #001-007(AN) enrolled in protocol #M34100-026(Faderl)	25
11-9-01		Record of Regulatory Agency Contact	Sean Bradley	Jackie Cinicola	confirmed receipt of briefing doc w/ S. Bradley and clarified remaining issues regarding end of Phase 2 meeting	
11-7-01	094	15 Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-068, Syncope, Chest tightness, Dyspnoea NOS, Postural hypotension and Herpes zoster, Patient #008-002(DRP) enrolled in protocol #M34100-025(Orlowski)	25
11-6-01		email to FDA	Sean Bradley	Jackie Cinicola	attachment of electronic version of briefing doc for PS341 meeting for 12/7. 18 copies were sent via FEDex to Dr. Pazdur	
11-6-01	093	End-of-Phase II Meeting Briefing Document	Richard Pazdur, MD	Jacqueline Cinicola	Briefing Document summarizing relevant PS-341 data and clinical development plan	25
11-5-01		email to FDA	Jackie Cinicola	Sean Bradley	correspondence about meeting for Dec 7th instead of Dec 6th	

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Date	Serial No.	Doc Type	To	From	Description	Location
11-2-01	091	15 Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-080, Syncope and Facial palsy, Patient #002-014(AHL) enrolled in protocol #M34100-025(Barlogie)	25
11-2-01	092	15 Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-081, Costochondritis, Patient #013-108(GHS) enrolled in protocol #M3101-028(Ryan)	25
10-25-01	090	Protocol Amendments: Change in Protocols	Richard Pazdur, MD	Meri Bloom	Amendment to both Phase II studies, M34100-024 M34100-025	25
10-22-01	089	15-Day IND Follow up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-064 of Ataxia NEC, Weakness amd Peripheral neuropathy NEC, Patient #014-002(RA) enrolled in protocol M34100-024(Irwin)	25
10-19-01	088	7-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-075, Weakness, Diarrhoea NOS and Cardio-respiratory arrest, Patient #003-027(BPB) enrolled in protocol M34100-025(Vescio)	24
10-16-01	087	15-Day IND Follow Up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-009 of Hypercalcaemia,Patient # 17(NGI) enrolled in protocol LCCC 9834(Orlowski)	24
10-15-01	086	15-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report S01-341-071, Cardiorespiratory arrest, Patient # 022-003(AAA)enrolled in protocol M34100-025(Berenson)	24

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Date	Serial No.	Doc Type	To	From	Description	Location
10-10-01	085	Protocol Amendment: Change in Protocol	Richard Pazdur, MD	Meri Bloom	Amendment to study, M34101-029	24
10-4-01	084	7-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-071, Cardiorespiratory arrest, Patient # 022-003(AAA) enrolled in Protocol M34100-025(Berenson)	24
10-3-01	082	Protocol Amendment: New Protocols	Richard Pazdur, MD	Meri Bloom	2 new protocols: Phase I/II #34101-033 and Phase I #34101-034	23
10-3-01	083	Protocol Amendment: New Investigators	Richard Pazdur, MD	Meri Bloom	024 - Raymond Alexanian, MD; Steven Limentani, MD; Paul G. Richardson, MD 025 - Steven Limentani, MD 026 - John G. Gribben; Ronald W. Takvorian; Kanti R. Rai 027 - David P. Ryan	24
10-2-01	080	15-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-063, Confusion and Hyponatremia, Patient # 001-007(AN) enrolled in Protocol M34100-026(Faderl)	23
10-2-01	081	15-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-064, Neurological symptoms NOS, Patient # 014-002(RA) enrolled in protocol M34100-024	23
9-28-01		FDA Fax	Libbie Mansell	Christy Wilson	Confirmation of the scheduled End of Phase II meeting for December 6, 2001	23

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Date	Serial No.	Doc Type	To	From	Description	Location
9-27-01	078	15-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-058, Pancreatitis NOS and Vomiting NOS, Patient #005-003(ARW) enrolled in Protocol M34100-025(Hussein)	23
9-27-01	079	15-Day IND Follow Up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-019 of Pul. edema Head., Hep. fun.abnormal, Pyrexia, Rigors, Thrombo. and Anae. , Pt#001 (ECB) enrolled in 025(Alsina)	23
9-26-01		Corresponse	Libbie Mansell, PhD	Dottie Pease	Aknoledgement for Fast Track Request made on September 20, 2001 submitted as Serial No. 073	23
9-25-01	076	7-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-061, Respiratory failure (exc neonatal), Patient #005-001 (SJM) enrolled in Protocol M34100-025(Hussein)	23
9-25-01	077	Annual Report	Richard Pazdur, MD	Meri Bloom	Annual Report for reporting period of September 1, 2000 through July 31, 2001	23
9-21-01	074	General Correspondence	Richard Pazdur, MD	Meri Bloom	Letter to authorize FDA to reference MPI's IND and subsequent sub. in review of IND to Agency by Dept. of GU Medical Oncology at University of Texas, MD Anderson for same product	22
9-21-01	075	General Correspondence	Richard Pazdur, MD	Meri Bloom	Letter to authorize FDA to reference MPI's IND and subsequent submissions for PS-341 in review of IND submitted to the Agency by Rush Cancer Institute in Chicago, IL for same product	22

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Date	Serial No.	Doc Type	To	From	Description	Location
9-20-01	073	Fast Track Designation Request	Richard Pazdur, MD	Libbie Mansell, PhD	Request for Fast Track Designation for PS 341	22
9-14-01	072	Protocol Amendment: Change in Protocols	Richard Pazdur, MD	Meri Bloom	Amendment to Phase I study, M34100-027	22
9-12-01	071	General Correspondence	Richard Pazdur, MD	Libbie Mansell, PhD	Formal request for Type B Meeting to discuss clinical development and registration plans for PS-341 **Missing from IND**	
8-31-01	070	15-Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-010, follow-up on initial report of Pneumonia NOS, Patient #17 (NGI) enrolled in Protocol LCCC 9834(Orlowski)	21
8-30-01	069	Protocol Amendment New Investigators	Richard Pazdur, MD	Meri Bloom	M34100-024, 1572 for Melissa Alsina, M.D. M34100-025, 1572 for Raymond Alexanian, M.D.	21
8-28-01	068	15-Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-019, report of Pul. edema NOS, Heachache NOS, Blood Disorder NOS, Hepatic function abnormal NOS, Pyrexia and Rigors, Patient #001 (ECB) enrolled in 025 (Alsina)	21
8-27-01	066	Protocol Amendment: Change in Protocol	Richard Pazdur, MD	Meri Bloom	M34100-024 - revised the inclusion criterion	20

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Date	Serial No.	Doc Type	To	From	Description	Location
8-27-01	067	Protocol Amendment: Change in Protocols	Richard Pazdur, MD	Meri Bloom	Amendment to both Phase II studies, M34100-025 and M34100-026	21
8-14-01	064	15-Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-006, previously coded as Rash Vesicular now changed to Herpes zoster, Patient #017 (NGI) enrolled in Protocol LCCC 9834(Orlowski)	20
8-14-01	065	15-Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-001, previously coded as Hepatic Function Abnormal now changed to Hepatitis NOS, Patient #014 (DGM) enrolled in Protocol LCCC 9834(Orlowski)	20
8-8-01	063	General Corespondence	Richard Pazdur, MD	Meri Bloom	Authorization for FDA to reference MLMN's IND in review of the IND submitted by the Lineberger Comprehensive Cancer Center	20
8-2-01	062	15-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-035, Arterial embolism limb, Patient #003-014 enrolled in Protocol M34100-025 (Berenson)	20
7-31-01	061	Protocol Amendment: New Investigators	Richard Pazdur, MD	Meri Bloom	M34100-024, 1572 for Barlogie; M34100-025, 1572s for Orlowski, Srkalovic; M34100-026, 1572 for Flinn	20
7-30-01	060	15-Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-019, Pulmonary oedema, Headache, Blood disorder, Hepatic function abnormal, Patient #001 (ECB) enrolled in Protocol M34100-025 (Alsina)	19

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Date	Serial No.	Doc Type	To	From	Description	Location
7-24-01	059	Protocol Amendment: Change in Protocol	Richard Pazdur, MD	Meri Bloom	M34100-025 - allowed enrollment of an additional 75 patients in a second cohort	19
7-9-01	058	15-Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Manufacturer's Report No. S01-341-001, Hepatic function abnormal, NOS, Patient #14 (DGM) enrolled in Protocol LCCC 9834 (Orlowski)	19
7-5-01	057	15-Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Manufacturer's Report No. S01-341-006, rash vesicular, Patient #17 (NGI) enrolled in Protocol LCCC 9834 (Orlowski)	19
6-29-01	056	7-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Manufacturer's Report No. S01-341-023, (Confusion aggravated, Ammonia increased), Patient #008 enrolled in Protocol M34100-025 (Irwin)	19
6-27-01	054	Protocol Amendment: New Investigators	Richard Pazdur, MD	Meri Bloom	M34100-024, 1572s for Berenson, Traynor; M34100-025, 1572s for Alsina, Berenson, Traynor; M34101-028, 1572 for Orlowski	19
6-27-01	055	15-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Manufacturer's Report No. S01-341-020, (Herpes zoster), Patient #001 enrolled in Protocol M34100-024 (Seigel)	19
6-22-01	053	15-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Manufacturer's Report No. S00-341-009, (dementia NOS), Patient #15 enrolled in Protocol DM98-194 (Papandreou)	19

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Date	Serial No.	Doc Type	To	From	Description	Location
6-21-01	052	15-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-019, Pulmonary oedema, Headache, Blood disorder, and Hepatic function abnormal, Patient #001 enrolled in Protocol M34100-025 (Alsina)	19
6-20-01	051	15-Day IND Follow-up Safety Report	Sean Bradley	Meri Bloom	Manufacturer's Report No. S00-341-028, death (previously reported serum sickness and dyspnoea NOS), Patient #06 enrolled in Protocol LCCC 9834 (Orlowski)	19
6-15-01	050	15-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Manufacturer's Report No. S01-341-010, Pneumonia NOS, Patient #017 (NGI) enrolled in Protocol No. LCCC 9834 (Orlowski)	19
6-14-01	049	7-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Mnfr's Report No. S01-341-019, Pulmonary oedema, Headache, Blood disorder, and Hepatic function abnormal, Patient #001 enrolled in Protocol M34100-025 (Alsina)	19
6-8-01	048	Protocol Amendment: Change in Protocol	Richard Pazdur, MD	Meri Bloom	M34100-026 - requiring additional blood samples, adding additional sites, clarifying dosing schedule, and several other clarifications and minor changes	19
6-5-01	047	Protocol Amendment: New Protocol	Richard Pazdur, MD	Meri Bloom	Protocol M34101-028 and 1572 for Ryan	18
5-9-01	046	Protocol Amendment: New Protocol	Richard Pazdur, MD	Meri Bloom	Protocol M34100-027 and 1572 for Ryan	18

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Date	Serial No.	Doc Type	To	From	Description	Location
5-8-01	045	Protocol Amendment: New Investigators	Richard Pazdur, MD	Meri Bloom	M34100-024, 1572s for Irwin, Richardson, Siegel; M34100-025, 1572s for Barlogie, Irwin, Richardson, Siegel	18
5-7-01	044	15-Day IND Initial Safety Report	Sean Bradley	Meri Bloom	Manufacturer's Report No. S01-341-009, hypercalcaemie, Patient #17 enrolled in Protocol LCCC 9834 (Orlowski)	17
4-26-01		Request for Formal Meeting	Richard Pazdur	Jackie Cinicola	Request for type a formal meeting to discuss the agency's remaining issues regarding the design for m34101-039 and confrim status of fast-track	
4-13-01	042	15-Day IND Follow-up Safety Report	Sean Bradley	Libbie Mansell, PhD	Manufacturer's Report No. S00-341-044, MI, cardiac failure congestive, depression suicidal, bacteremia, Patient #13 enrolled in Protocol LCCC 9834 (Orlowski)	17
4-13-01	043	Protocol Amendment: Change in Protocol	Richard Pazdur, MD	Meri Bloom	M34100-024 and M34100-025 - required PK analyses for patients at Dana Farber and added Independent Review Committee for efficacy review; 1572 for Jagannath	17
4-4-01		Contact Report	Sean Bradley	Meri Bloom	Patient #6 in LCCC-9834 passed away prior to receiving drug on a compassionate use basis for protocol M34101-030	17
4-2-01	041	15-Day IND Initial Safety Report	Sean Bradley	Libbie Mansell, PhD	Manufacturer's Report No. S01-341-006, rash vesicular, urinary retention, Patient #17 enrolled in Protocol LCCC 9834 (Orlowski)	17

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Date	Serial No.	Doc Type	To	From	Description	Location
3-19-01		FDA FAX	Libbie Mansell, PhD	Sean Bradley	Responses to End-of-Phase 1 Meeting Package submitted 2-21-01 and new Biopharm comments	16
3-19-01		FDA FAX	Libbie Mansell, PhD	Sean Bradley	Agency's list of attendees for End-of-Phase 1 Meeting scheduled for 3-22-01	16
3-19-01	040	15-Day IND Follow-up Safety Report	Sean Bradley	Libbie Mansell, PhD	Manufactuer's Report No. S00-341-001 (old #20001), small intestinal obstruction NOS, Patient #33 enrolled in Protocol 98-104 (Aghajanian)	16
3-14-01	039	Protocol Amendment: New Protocol	Richard Pazdur, MD	Libbie Mansell, PhD	Protocol M34100-029 and 1572 for Papandreou	16
3-9-01		Protocol Amendment: New Protocol	Richard Pazdur, MD	Libbie Mansell, PhD	Protocol M34100-024 and 1572 for Richardson; Protocol M34101-026 and 1572 for Faderl	16
3-2-01	037	15-Day IND Follow-up Safety Report	Sean Bradley	Libbie Mansell, PhD	Manufacturer's Report No. S01-341-001, hepatic function abnormal, Patient #14 enrolled in Protocol LCCC 9834 (Orlowski)	15
3-1-01		MPI fax	Sean Bradley	Libbie Mansell, PhD	Request for a protocol exemption to permit compassionate treatment of Patient #6 (SAE) at LCCC by Dr. Orlowski	15

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Date	Serial No.	Doc Type	To	From	Description	Location
2-27-01	036	Information Amendment: CMC	Richard Pazdur, MD	Libbie Mansell, PhD	Stability data and recertification of bulk active drug substance lot # 970087; change in Drug Product Manufacture; cross reference authorization to the FDA from the NCI	15
2-22-01	035	Serial No. Voided				
2-21-01	033	General Corespondence	Richard Pazdur, MD	Libbie Mansell, PhD	End-of-Phase 1 Meeting briefing document	15
2-21-01	034	General Corespondence	Sean Bradley	Libbie Mansell, PhD	15 desk copies of the End-of-Phase 1 Meeting briefing document	15
2-5-01		Contact Report	Libbie Mansell, PhD	Sean Bradley	Discussed what is required to request authorization to treat a patient on a compassionate use basis	15
1-23-01	032	General Corespondence	Richard Pazdur, MD	Libbie Mansell, PhD	Request for an End-of -Phase 1 meeting to discuss proposed clinical development plan	14
1-17-01		Contact Report	Sean Bradley	Libbie Mansell, PhD	Questioned sending out Protocol M34100-024 to sites prior to the EOP1 meeting. FDA would prefer if we waited until after the EOP1 meeting.	14

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Date	Serial No.	Doc Type	To	From	Description	Location
1-16-01	031	15-Day IND Initial Safety Report	Sean Bradley	Libbie Mansell, PhD	Manufacturer's Report No. S01-341-001, hepatic function abnormal, Patient #14 enrolled in Protocol LCCC-9834 (Orlowski)	14
12-20-00	030	Protocol Amendment: New Protocol	Sean Bradley	Marian Smith	Protocol M34100-025 and NCI cross reference letter for PS-341 IND 58,443	14
12-7-00	029	IND Safety Report (Animals)	Sean Bradley	Marian Smith	Re-examination of nerve sections from Study # 6837-109 (cynomolgus monkeys) showed evidence of nerve fiber degeneration at the high dose level.	14
11-22-00		MPI FAX	Sean Bradley	Marian Smith	Signed copy of Form FDA 1571 for Serial No. 027, which was previously submitted without a signature.	14
11-22-00		General Corespondence	Marian Smith	Sherry Ansher	Copy of cross-reference authorization letter to the NCI-sponsored IND # 58,443	14
11-20-00		MPI FAX	Sean Bradley	Marian Smith	2nd Fax of Serial No. 028, 7-Day IND Safety Report, Manufacturer's Report No. S00-341-044	14
11-20-00		General Corespondence	Sherry Ansher	Marian Smith	Copy of (10/2000) Annual Report sent to NCI	14

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Date	Serial No.	Doc Type	To	From	Description	Location
11-20-00		MPI FAX	Sean Bradley	Marian Smith	Signed copy of Form FDA 1571 for Serial No. 026, which was previously submitted without a signature.	14
11-20-00		General Corespondence	Peter Elliott	Sherry Ansher	Copy of the NCI's 2000 Annual Report submitted to FDA on 10-19-000	14
11-20-00	028	7-Day IND Initial Safety Report	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-044, (MI, cardiac failure, suicide attempt, bacterial infection), Patient # 13 enrolled in LCCC-9834 (Orlowski)	14
11-15-00	027	15-Day IND Initial Safety Report	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-043, hypokalemia, Patient # 13 enrolled in Protocol No. LCCC 9834 (Orlowski)	14
11-9-00	026	15-Day IND Initial Safety Report	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-040, hypotension and atrial fibrillation, Patient #40 enrolled in Protocol No. DM98-194 (Papandreou)	14
11-2-00	025	IND Submission	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-031, dyspnoea, dysphagia, aggravated renal failure, Patient # 8 enrolled in Protocol No. LCCC 9834 (Orlowski)	14
10-25-00	024	15-Day IND Initial Safety Report	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-034, hyponatraemia, Patient # 10 enrolled in Protocol No. LCCC 9834 (Orlowski)	13

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Date	Serial No.	Doc Type	To	From	Description	Location
10-23-00	023	Annual Report	Sean Bradley	Marian Smith	August 29, 1999 - August 31, 2000 Safety Follow-up Information (S00-341-028, S00-341-029 and S00-341-031)	13
9-14-00	022	15-Day IND Follow-up Safety Report	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-001, ileus, Patient # 33 and No. S00-341-011, neuropathy peripheral, Patient #31 enrolled in Protocol No. 98-104 (Aghajanian)	13
9-6-00	021	15-Day IND Follow-up Safety Report	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-028, serum sickness, dyspnoea, Patient # 6 enrolled in Protocol No. LCCC 9834 (Orlowski)	13
8-29-00	020	15-Day IND Initial Safety Report	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-033, neuropathy peripheral, Patient # 35 enrolled in Protocol No. 98-104 (Aghajanian)	13
8-23-00	019	Protocol Amendment: New Investigator	Sean Bradley	Marian Smith	1572 for Anderson, Protocol Exemption for LCCC 9834 at Dana Farber Cancer Institute	13
8-22-00	018	15-Day IND Initial Safety Report	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-031, dyspnoea, dysphagia, aggravated renal failure, Patient # 8 enrolled in Protocol No. LCCC 9834 (Orlowski)	13
8-8-00	017	15-Day IND Initial Safety Report	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-030, fever, rigors, Patient # 39 enrolled in Protocol No. DM98-194 (Papandreou)	13

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Date	Serial No.	Doc Type	To	From	Description	Location
8-1-00	016	15-Day IND Initial Safety Report	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-028, rash, dyspnoea, arthropathy, Patient # 6 enrolled in Protocol No. LCCC 9834 (Orlowski)I	13
7-7-00		Contact Report	Sean Bradley	Marian Smith	Inquired the status of any clinical comments on Dr. Soignet's Protocol No. 00-31A(1). No comments available, but received approval to start the protocol.	13
7-5-00	015	15-Day IND Follow-up Safety Report	Dotti Pease	Marian Smith	Manufacturer's Report No. S00-341-002, fever, granulocytopenia, leucopenia, Patient # 27 enrolled in Protocol No. 98-104 (Aghajanian)	13
6-26-00		Contact Report	Sean Bradley	Marian Smith	Confirm FDA's receipt of Serial No. 013 containing Dr. Soignet's Protocol No. 00-31A(1) and inquired as to when we can begin the protocol.	13
6-26-00	014	15-Day IND Initial Safety Report	Sean Bradley	Marian Smith	Manufacturer's Report No. S00-341-011, neuropathy peripheral, Patient # 31 enrolled in Protocol No. 98-104 (Aghajanian)	13
6-21-00	013	Protocol Amendment: New Protocol	Sean Bradley	Marian Smith	Protocol No. 00-31A (1) and 1572 for Soignet at MSKCC	13
6-20-00		Contact Report	Sean Bradley	Marian Smith	Discussed the submission of Dr. Soignet's Protocol 00-31A(1). It will be reviewed as a new protocol by the FDA.	13

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Date	Serial No.	Doc Type	To	From	Description	Location
6-7-00		Acknowledgement Letter	Marian Smith	Dotti Pease	Acknowledge receipt of Serial No. 010 (4-19-00) notifying FDA of corporate name change from LeukoSite to Millennium	13
6-6-00		FDA FAX	Marian Smith	Sean Bradley	Request sent on 6-5-00 for a Protocol Exemption to treat a patient by Dr. Ken Anderson at Dana Farber has been granted	13
6-5-00		MPI fax	Dotti Pease	Marian Smith	Protocol Excemption Request to permit compassionate treatment of a patient at Dana Farber by Dr. Ken Anderson according to Protocol LCCC 9834	13
6-5-00		Contact Report	Dotti Pease	Marian Smith	Inquired what is required to treat a patient on a compassionate use basis; the Protocol Excemption was approved by Sean Bradley for this patient to be treated at Dana Farber	13
5-30-00	012	General Corespondence	Dotti Pease	Marian Smith	Reporting of two AEs that do not meet the criteria for expedited IND Safety reporting; S00-341-008 (forgetfulness) and S00-341-009 (confusion, inappropriate behavior)	13
5-5-00	011	15-Day IND Initial Safety Report	Dotti Pease	Marian Smith	Mnfr's Rpt No. S00-341-002, fever, leucopenia granulocytopenia, Pt. # 27, 98-104, (Aghajanian) and No. S00-341-006, hypotension postural, tachycardia, Pt. # 31, 98-194 (Papandreou)	13
4-19-00	010	15-Day IND Initial Safety Report	Dotti Pease	Marian Smith	Manufacturer's Report No. 20001, Ileus, Patient # SD enrolled in Protocol No. 98-104 (Aghajanian); corporate name change from LeukoSite to Millennium	13

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Date	Serial No.	Doc Type	To	From	Description	Location
4-14-00		MPI fax	Dotti Pease	Marian Smith	Fax of Serial No. 010, Manufacturer's Report No. 20001, Ileus, Patient # SD enrolled in Protocol No. 98-104 (Aghajanian)	13
11-29-99		letter	Peter Elliott	Dotti Pease	Letter from FDA aknowlegding transfer of ownership of IND 56, 515 from ProScript, Inc. to LeukoSite, Inc.	12
11-24-99		phone contact	Loretta Arscott	Anne Maire Gregg	Telephone contact to Loretta Arscott in regard to advice about the structure and strategy for the development of a Phase II program	12
10-22-99	009	IND Submission	Robert Justice, MD	Peter Elliott	Annual Report for 1999	12
9-2-99		letter	Robert Justice, MD	Peter Elliott	Letter to Dr. Justice regarding continuing treatment with higher doses of PS-341 (beyond top dose) at clinical sites MDACC and MSKCC	11
7-20-99		letter	Christy Wilson, CSO	Peter Elliott	Letter to Christy Wilson to reply to FDA's comments regarding proposed Phase I trial of PS-341/CPT-11	11
6-22-99		fax	Peter Elliott	Christy Wilson, CSO	Facsimile from Christy Wilson providing FDA's clarifications of remarks made to Proscript's questions posed in 5/7/99 mtg. package for PS-341	11

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Date	Serial No.	Doc Type	To	From	Description	Location
6-18-99		letter	Robert Justice, MD	Peter Elliott	Letter to Dr. Justice providing ProScript's proposal to 6/16/99 FDA's comments for PS-341 and request to postpone meeting with Agency on Tuesday, June 22, 1999.	11
6-16-99		fax	Peter Elliott	Christy Wilson, CSO	Facsimile from Christy Wilson regarding FDA's responses to questions posed by ProScript in 5/7/99 mtg. package for PS-341	11
6-16-99		letter	Robert Justice, MD	Peter Elliott	letter to Dr. Justice regarding the agenda for the 5/22/99 meeting scheduled to discuss proposed Phasel trial of PS-341/CPT-11	11
5-25-99		letter	Robert Justice, MD	Peter Elliott	Letter to Dr. Justice confirming meeting of 6/22/99	11
5-25-99	008	IND Submission	Robert Justice, MD	Peter Elliott	New protocol submission- "Phase I Evaluation of PS-341 in Patients with Hematologic Malignancies" and New Investigator (Robert Orlowski)	11
5-17-99		letter	Christy Wilson, CSO	Marlene Booth	Additional copies of the 5/7/99 premeting package	10
5-14-99		fax	Marlene Booth	Christy Wilson, CSO	Confirmation of End-of-Phase I (Guidance) meeting scheduled for June 22, 1999	10

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Date	Serial No.	Doc Type	To	From	Description	Location
5-13-99		phone contact	Marlene Booth	Christy Wilson, CSO	FDA scheduled meeting for Tuesday 6/22/99	10
5-12-99		phone contact	Marlene Booth	Christy Wilson, CSO	Confirmation of reciept of revised May 7, 199 meeting package and gave proposed time for meeting -June 18 or 22	10
5-10-99	007	Response To FDA Request for Information	Robert Justice, MD	Marlene Booth	Response to FDA telefaxes dated 4/21/99 regarding Serial No. 006	10
5-7-99		Letter	Christy Wilson, CSO	Marlene Booth	Letter to Christy Wilson provideing 6 replacement copies of May 7, 1999 replacing April 30, 1999.	9
5-7-99		Meeting Package	Robert Justice, MD	Marlene Booth	Pre-meeting package for PS-341/CPT-11 Phase I protocol discussion	9
5-4-99		fax	Marlene Booth	Christy Wilson, CSO	Telefax from FDA clarifying their April 12, 1999 request (Serial No. 006 CMC review) for a tighter Content Uniformity specification	9
4-30-99		Request for Meeting Package	Robert Justice, MD	Marlene Booth	Request for meeting to discuss PS-341/CPT-11 Phase I protocol (University of North Carolina)	9

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Date	Serial No.	Doc Type	To	From	Description	Location
4-21-99		fax	Marlene Booth	Christy Wilson, CSO	Telefax from FDA with coments regarding Serial No. 006 -chemistry review.	9
4-16-99		phone contact	Marlene Booth	Christy Wilson, CSO	Follow-up telephone conversation with Christy Wilson delivering the amendment (Serial No. 006) to the CMC reviewer.	9
4-12-99	006	Information Amendment: CMC	Robert Justice, MD	Marlene Booth	Information ammendment containing CMC information regarding the PS-341 lyophilized finished product and stability update for the GMP bulk active drug substance and liquid formulation	8
4-1-99	005	Submission	Robert Justice, MD	Marlene Booth	Protocol Ammendment:Change in Protocol for Phase I Study 98-104 at MSKCC (minor corrections and clarifications)	7
3-8-99	004	Submission	Robert Justice, MD	Marlene Booth	Protocol Ammendment:Change in Protocolfor Phase I Study 98-194 at MDACC to clarify the intened doses administration timeline and to provide for earlier dose escalation	6
2-12-99		letter	Robert Justice, MD	Marlene Booth	Cross-reference authorization letter for NCI IND	5
2-4-99	003	Information Amendment: Pharacology/Toxicology	Robert Justice, MD	Marlene Booth	Submission of two final preclinical study reports -NCI Multidose Study in Rats and NCI Cardiotoxicity Study in Monkeys	5

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Date	Serial No.	Doc Type	To	From	Description	Location
12-18-98	002	Protocol Amendment: New Protocol/New Investigator information	Robert Justice, MD	Marlene Booth	Protocol Amendment: New Protocol for Phase I Study 98-104 at the Memorial Sloan-Kettering Cancer Center; New Investigator information for Dr. Carol Aghajanian	4
10-1-98	001	Prot Amend: Change in Protocol and Info Amend: Pharm/Tox	Robert Justice, MD	Marlene Booth	IND ammendment in response to Aug 26/98 telefax, including revised clinical protocol and 20S proteasome activity SOPs. Copies for your reference	3
9-17-98		phone contact	Debbie Catterson	Marlene Booth	Phone call to FDA request to extend submission date for serial No. 001 to October 2nd	3
8-27-98		phone contact	Debbie Catterson	Marlene Booth	Phone call to D. Catterson to discuss Aug 26/98 telefax; minor deficiencies to be addressed by protocol revision; verbal approval of IND received	3
8-26-98		fax	Debbie Catterson	Marlene Booth	telefax from D Catterson listing 8 minor IND deficiencies and requesting a telephone discussion after ProScript reviews deficiencies.	3
8-21-98		fax	Dr. Cheng Yi Liang	Marlene Booth	Fax to Dr. Liang regarding the CMC requested data for GMP Bulk Active Drug Subtance Lot 970087	3
8-21-98		phone contact	Dr. Cheng Yi Liang	Marlene Booth	Phone call from Dr. Liang regarding copies of CMC test data	3

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Date	Serial No.	Doc Type	To	From	Description	Location
8-21-98		fax	Debbie Catterson	Marlene Booth	Fax to Debbie informing her that a telefax was sent to Dr. Liang regarding the CMC requested data for GMP Bulk Active Drug Subtance Lot 970087	3
8-20-98		phone contact	Marlene Booth	Dr. Hua Zheng	Phone call from Dr. Zheng regarding clarification regarding source documentation for the pharmacology/toxicology review of IND	3
8-10-98		Acknowledge receipt of IND	Marlene Booth	Debra Catterson for	IND acknowledgment receipt and IND assignment number 56, 515	3
7-28-98		phone contact	Central Doc. Rm.	Marlene Booth	phone call to Central Document Room to confirm receipt of IND and obtain assigned IND number 56,515	3
7-24-98	000	IND Submission	Robert Justice, MD	Marlene Booth	PS-341 Investigational New Drug Submission (11 volumes)	2.1-2.11
6-30-98		pre-IND	Debbie Catterson	Marlene Booth	response to FDA's June 25/98 comments	1
6-26-98		phone contact	Debbie Catterson	Marlene Booth	phone call confirming cancelation of 6/29/98 pre-IND	1

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Date	Serial No.	Doc Type	To	From	Description	Location
6-25-98		FDA Facismile	Marlene Booth	Debbie Catterson	Facismile from FDA with comments relating to review of pre-IND meeting package	1
6-25-98		phone contact	Marlene Booth	Debbie Catterson	phone conversation stating that the pre-IND meeting was not needed-FDA had no questions that necessitate a meeting	1
6-24-98		letter	Debbie Catterson	Marlene Booth	fax to FDA submitting revised Phase I clinical outline	1
6-9-98		phone contact	Dottie Pease	Marlene Booth	phone call from Pease regarding pre-IND meeting	1
6-5-98		pre-IND Meeting Package	Dottie Pease	Marlene Booth	additional three copies of pre-IND meeting package, revised list of participants and draft agenda	1
6-3-98		phone contact	Fran Rowland	Marlene Booth	called to clarify which binders/colors to use for the non-chemistry volume review copies	1
6-1-98		phone contact	Christy Wilson	Marlene Booth	regarding the pre-IND meeting scheduled for 6/29/98. Left message with Dottie Pease regarding logistics of the meeting	1

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Date	Serial No.	Doc Type	To	From	Description	Location
5-28-98		pre-IND Meeting Package	Pease	Booth	Six copies of the pre-IND meeting package sent to Dottie Pease at FDA	1
5-15-98		Request for Pre-IND Meeting	Robert DeLap	Marlene Booth	Request for pre-IND meeting	1
11-3-97		phone contact	Marlene Booth	Dottie Pease	Acknowledgment of meeting request withdrawl	1
10-29-97		phone contact	Marlene	Dottie Pease	phone call regarding proposed meeting date of November 12th or 13th, new FDA meeting format and requesting outline for planned Phase I clinical study	1
10-22-97		Request for Meeting	Robert DeLap, MD	Marlene Booth	Called to request a meeting with the Agency to review protocol design of the repeat dose toxicity and study in monkeys	1
7-24-97		phone contact	Dottie Pease	Marlene Booth	Called to discuss final formulation concentration and vial size/fill volume	1
7-2-97		fax	Dotti Pease	Marlene Booth	**FDA meeting minutes of 6-13-97 meeting. Minutes not included in book 1. Need to search for copy in other sources.**	1

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Date	Serial No.	Doc Type	To	From	Description	Location
6-26-97		letter	Robert DeLap, MD	Marlene Booth	Prosscript meeting minutes discussing the manufacturing of PS-341 GMP bulk active drug substance	1
6-13-97		Memorandum of Meeting	Marlene Booth		Internal memo discussing adequacy of proposed CMC workup for IND, paricularly bulk active drug substance manufacture	1
6-13-97		Internal Meeting package	Marlene Booth		Agenda for meeting scheduled for June 13, 1997 with slides discussing synthesis and manufacturing plan of PS-341	1
6-9-97		Phone contact	Marlene Booth	Dottie Pease	Call from FDA regarding meeting confirmation and revised agenda received, and list of attendees to the June 13th meeting	1
6-6-97		fax	Dottie Pease	Marlene Booth	Fax confirming the 6/13/97 meeting to discuss the manufacturing of PS-341-GLP Step 4 intermediate	1
5-19-97		phone contact	Marlene Booth	Dottie Pease	Scheduled meeting for 6/13/97-discussion of manufacutring of PS-341	1
5-14-97		phone contact	Dottie Pease	Marlene Booth	Phone call confirming FDA receipt of the telefax and also to inform Proscript that FDA still needs to assign a reviewer before they can meet with ProScript.	1

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Date	Serial No.	Doc Type	To	From	Description	Location
5-13-97		fax	Dottie Pease	Marlene Booth	Response to FDA request for information in response to May 2, 1997 fax, in preparation for CMC meeting	1
5-13-97		fax	Dottie Pease	Marlene Booth	**Response to FDA request for meeting and information. Twenty-four page response is missing from book 1. Need to look at other sources for information.**	1
5-2-97		fax	Marlene Booth	Dottie Pease	FDA questions regarding PS-341 that will be reviewed	1
4-25-97		fax	Dorothy Pease	Marlene Booth	Request a meeting with the Agency to discuss the manufacture of PS-341 bulk active drug substance	1

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Date	Serial No.	Doc Type	To	From	Description	Location